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Harvard backs off recombinant DNA

Harvard University's decision (see *Nature* 27 November) not to become a minority partner in a commercial venture to exploit recombinant DNA technology will have more than merely parochial consequences. Although it is probably no longer the case that universities in the United States and even elsewhere take their cues from what Harvard does, the public agony in which the university has come to its decision cannot fail to be instructive. Naturally, different universities will interpret events since the beginning of the term in October in different ways. Some will say that what has happened only goes to show that Harvard is the stuffy, or indecisive, place they have always supposed it to be. Others will be confirmed in their conviction that commerce does not mix with academic life. Harvard itself may be surprised to discover that the issue which it has been debating for the past two months has stirred up other and less tractable issues that will themselves keep both the administration and the faculty on tenterhooks for some time to come. What are the circumstances in which academic institutions may properly take a commercial interest in the academic research of members of their faculties? What limits should there be on the entrepreneurial interests of members of university staffs? And what arrangements do universities need for regulating these questions? Plainly there is a long agenda ahead.

The events of the past few months at Harvard are not without interest in themselves. The tale goes back to the beginning of this year, when small companies for the exploitation of recombinant DNA were springing up all over the place. One of the Harvard faculty, Professor W. Gilbert, was already a prominent member of the company called Biogen which held a press conference in Boston to make public its plans for using cloning techniques for the manufacture of interferon. Some of Gilbert's Harvard colleagues were even then disturbed at the implications of his involvement with a commercial company in such a prominent way. For although Harvard is probably more rigorous than most other distinguished universities in making sure that members of the faculty do not spend more than a limited proportion of their time on extramural activities, people were concerned that the work of one of their most creative colleagues (Gilbert shared this year's Nobel prize for chemistry) might in future be less freely shared within the university. The proposal that the university should become a minority shareholder (without paying for its shares) in a commercial company to exploit recombinant DNA techniques was at least in part inspired by the hope of doing the same thing while benefiting the university in some tangible way. The plan was that research carried out in Professor Mark Ptashne's laboratory should be exploited by a new company financed by venture capital, managed and housed separately from the university but from which the university would derive a substantial income. The university administration seems from the beginning to have been keen on the prospect of an alternative source of income. If it had seemed less greedy, the Faculty of Arts and Sciences might have been less quick to take fright. But, in the end, the proposal foundered on the faculty's misgivings. For a time, Cambridge (Massachusetts) seems to have been thick with anxiety about secrecy, academic freedom and the like, but perhaps the clinching argument against the commercial venture was that the university could not, in the arrangements proposed, avoid discriminating in favour of those on whose research its financial prospects seemed to depend. In a college of supposed equals, some would be more equal than others.

Such issues are familiar stimulants of cant, and even Harvard's

internal argument appears not to have been entirely blameless in this respect. Talk of academic freedom jeopardized would have been more persuasive if those professing concern for this lofty cause had been able to specify what specific freedom might be lost. It is also important to acknowledge that both Gilbert and Ptashne have been entirely open with their colleagues (and with outsiders) about their motives. Indeed, it is ironical that Ptashne, the latecomer in commercial matters, should now be widely identified with a project which the university has flirted with but dropped. Meanwhile, the serious questions the Harvard faculty has been asking about the entrepreneurial role of universities, or of members of their faculties, remain unanswered.

There is one important respect in which the issue is already decided. Since 1975 and the change in that year in the rules for the ownership of patents deriving from research carried out with research grants from the National Institutes of Health, Harvard (like other American universities) has become a substantial patent holder. The new rule is that universities can acquire the rights in patentable inventions arising within their laboratories if they can somehow persuade those responsible to apply for legal protection. Experience, not only at Harvard, has shown that little benefit accrues to anybody — the university or even the public — if a patent holder simply sits on his patent. Exploitation requires flair, finance and a keen sense for dollar bills (or for whatever other currency may be concerned). Even well organized universities in the United States are probably less commercially effective at making money out of patents than would be strictly commercial organizations. (In Britain, where the National Research Development Corporation is the public patent holder on behalf of academics and their universities, complaints are common that more might be done to make money out of people's cleverness.) In a sense, Harvard's proposed venture into biotechnology was merely an extension of its established role as a patent holder. On the face of things, moreover, the university was not being asked to back the new company with funds of its own. Yet there is a difference of principle between earning royalties on a set of patents and being a shareholder in a company. Substantial shareholders, however cheaply acquired their equity, must take a serious interest in the management of their companies — and must be prepared, when things go wrong, to step in quickly with extra funds. There is no reason to suppose that Harvard would be any more able to shoulder these responsibilities than other universities. Indeed, given the way in which at Harvard the administration's power of decision is rightly blunted by the veto powers distributed among the several faculties, Harvard is not everybody's favourite business partner.

There is also force in the argument, much canvassed in the past two months, that a university cannot enter into a risk-taking business partnership with some section of its own faculty without resolving the obvious difficulties in advance. Whatever the terms of the partnership, success is certain to engender a pattern of importance within the faculty that reflects considerations other than academic merit. Ptashne, no doubt, would argue that arrangements could have been made for avoiding such problems; a faculty as vigilant as that at Harvard is unlikely to have allowed the university administration to favour him and his immediate colleagues with extra space and resources, however successful the new venture. The more serious problems would have arisen if the venture seemed to be failing. Would it then have been possible for either partner to have behaved as if only academic business mattered? This is the sense in which joint involvement in a



commercial company differs from the sale and exploitation of patent rights, however profitable. The circumstances in which universities may properly set up commercial companies to exploit work of their faculty members are thus easily defined. Success must not depend on future promise, and there must be no foreseeable circumstances in which an academic will feel compelled to function as the research or even marketing director of a company if things should start going wrong. The academic freedom most at risk is that of the academics who are most directly involved.

A further difficulty, which persists even though the university has said no to its commercial venture, is that the laboratories of individual professors will continue to be sources of research with entrepreneurial potential. Even if Ptashne decides not to go ahead independently of the university, Gilbert as a member of Biogen will no doubt remain on the lookout for ideas that can be exploited commercially. No doubt he will be as anxious as his academic colleagues that he should never be open to the charge of making inequitable use of his position, and of his access to university facilities. The most immediate cause for concern must be the graduate students working in his and other people's laboratories. Graduate students are called students because the university to which they belong is responsible for their further education, and the formalities of registration for higher degrees reflect that understanding. There is, however, an obvious danger that in the present excitement about recombinant DNA, the Gilberts and Ptashnes of this world, but also their graduate students, will put commerce before education. Having said no to the university's proposal, the Harvard faculty now has no choice

but to make more explicit its corporate responsibility for graduate students.

In the long run, Harvard will find it necessary to take a further and more painful step. A university is a kind of club of academics, kept together by mutual respect. Academics' external interests are by no means always destructive of that spirit. Academics who serve their governments, who write good literature or who add to the public enlightenment in other ways, often by doing so enhance the esteem in which they are held by their colleagues and in which their university is held by the wider public. Naturally, most universities have explicit rules for making sure that academics are not so caught up in extramural activities that they have insufficient time for their students, but there are other less formal constraints to be reckoned with. The worst offence an academic can commit against his colleagues is to abuse his position as a member of his academic club.

Yet universities (like other kinds of clubs) differ in their unspoken definitions of what constitutes abuse. Even at Harvard, it seems to be accepted that within reason people should be able to advise commercial companies. The problem of the faculty member as entrepreneur in the exploitation of his own research seems not previously to have arisen as sharply as in the past few months. (At some other universities, it is commonplace.) The Harvard faculty seems to have been taken by surprise. It has only itself to blame for not having made its unspoken rules explicit long ago. What it needs now is a general understanding that people's extramural activities and the financial gains therefrom are fully disclosed within the faculty, and are proper material for faculty discussion and even decision.

How not to run a public monopoly

The British government's proposals for the reorganization of the British Post Office (see *Nature* 27 November) seem calculated to win the worst of all possible worlds for everybody concerned, but the hapless users of telecommunications systems in particular. The chief purpose of the bill soon to begin its passage through the House of Commons is to separate the postal service from the rest of this nationalized industry, and there is no argument about the wisdom of that course. Indeed, for more than a decade the Post Office has been organized internally as if it were two organizations — a loss-making postal service and a money-making telecommunications service. Three years ago, Sir Charles Carter's committee on the problem told the government that it should go the whole way along this road, and formally split the two parts of the business. Everybody agrees that this should be done. But how?

The issue is important for several reasons, but not least because of the technical importance of the telecommunications industry to the wealth (and even the health) of a modern state. For far too long, Britain has been badly served by the electronic half of the Post Office. When other countries were installing modern and increasingly versatile telephone systems, would-be British users were forced to wait, often for more than a year, for the privilege of renting an antiquated handset. From time to time, bursts of excitement would nevertheless filter out of the British government's public relations network, explaining that this or that new miracle was about to be brought into service. In the late 1950s, for example, the "world's first" electronic exchange was being praised to the skies. Unfortunately, it never worked. More recently, with the new internal arrangements at the Post Office, there has been discerned a sense of realism. The managers of the telecommunications network have plainly begun to come to grips with the nature of their business. Keeping up with the Joneses (or the Japanese) technically is much more difficult than it seemed in the old days, when the Post Office would draft a specification for some new system and farm out the development and manufacture of the equipment to a cosy cartel of manufacturers. But the technical problems are not even half the battle. Managing a complicated telecommunications network is hair-raising.

Meeting the cost of developing a modern network is an exercise in high finance.

In all the recent discussions about the British Post Office, it has been plain that the crucial question to decide is where the line should be drawn between the public monopoly and private enterprise. Again, there is no dispute about the good sense of monopoly ownership and management of the telecommunications network. Every modern state has been driven to recognize as much. The questions that arise concern the definition of the monopoly at its margins, and the arrangements for its regulation. In Britain, there has been a lot of excited talk (encouraged by Sir Keith Joseph the industry minister) about the shading of the telecommunications monopoly. There are two respects in which this might with advantage be done. There is a need that those who rent access to telephone lines or other channels of communication should be allowed to attach to them whatever terminal equipment they choose, provided it is technically compatible with the network (and also legal). For no single organization, however competent (and British Telecom has a great deal of depressing history to live down), can hope itself to supply everything that users of the network are likely to need in the decades ahead. But the network (whatever it is) should also be usable by people prepared to offer services that the monopolists themselves choose not to offer.

Sir Keith Joseph's reorganization bill fudges both these issues. British Telecom will not have an exclusive right to supply all terminal equipment, but it will be dragged in if an attachment has to be serviced, while the minister's own civil service will assume some of the responsibility for telling what attachments can be attached. And yes, there will be arrangements for letting private people use the network for providing novel services, but again the details are not worked out and the minister's civil servants will again decide. The consequences of these un-Tory compromises are potentially disastrous. Neither British Telecom, the users of the network nor manufacturers of equipment will know where they stand, but crucial decisions about the development will return from whence they came several years ago — to the civil service.

Anti-trust waiver for industrial research

Justice blesses inter-company collaboration

Washington

The US Department of Justice has given the green light to research programmes jointly sponsored by two or more private companies, arguing that in general — and provided certain criteria are respected — these should not conflict with the country's strict anti-trust legislation.

Uncertainty about the implications of anti-trust laws is often cited by US business as an obstacle to greater cooperation in research. Their concern is that, once a joint research project has been agreed and set up, they may be accused of violating legislation developed to ensure maximum competitiveness between rival companies.

As a result, anti-trust legislation was closely studied during last year's domestic policy review of industrial innovation, carried out under the auspices of the Office of Science and Technology Policy (OSTP) and the Department of Commerce.

Announcing the results of this review last November, President Carter said that by spurring competition, anti-trust policies could provide a stimulant to innovation, but he added that in some cases — such as research — industrial cooperation might have clear social and economic benefits for the country.

"Unfortunately our anti-trust laws are often mistakenly viewed as preventing all cooperative activity", Mr Carter said. And, rather than proposing any change to the law, he instructed the Department of Justice to publish a guide explaining its interpretation of the laws that already exist.

The guide was finally published in Washington last week. It is now being closely studied by companies and research associations contemplating joint research ventures, since, although not a legally binding document, it indicates how the department is likely to react to particular arrangements.

The guide should also help to clear the way for the creation of so-called Cooperative Generic Technology (COGENT) centres. These have been proposed by the Department of Commerce and the National Science Foundation, jointly funded by government and various industrial companies in fields such as welding, lubrication and powdered metal processing. The idea was approved by Congress in recently passed legislation.

The guide points out that there are various ways in which joint research

ventures could involve anti-trust considerations. For example, they could lead to market-dominating technology, to unfair collaboration between commercial competitors or to restrictive agreements on the use of research results.

Each of these might restrict open competition. At the same time, the guide says, competitiveness is a source of increased innovation — while innovation itself is a basis for commercial competitiveness — so that to discourage the one is to discourage the other.

So the challenge is to develop an arrangement that will maximize the rate of innovation (and hence competitiveness) by permitting research that would not otherwise be carried out, but in a way that does not give one or more companies an

unfair advantage over others in the same field.

Rather than providing any hard and fast rules on how this should be done, the Department of Justice offers general guidance on how it would probably interpret existing statutes in particular situations. And it includes eight hypothetical case studies intended to illuminate its position.

As far as basic research is concerned, the department says that cooperation is unlikely to be much of a problem, since the competitive significance of the research is likely to be largely speculative and, as the results would be published in the open literature, there should be few problems about equal access by other companies.

David Dickson

Science foundation all set at last

Washington

As widely expected, the US National Science Foundation (NSF) has announced its intention to set up a new directorate for engineering, and to distribute responsibility for applied science — at present administered jointly with engineering — across the foundation's basic science directorates.

Announcing the planned reorganization to members of the National Science Board last week, NSF's director-designate, Dr John Slaughter, said that creating the new directorate meant that the foundation intended to seek more resources for the engineering disciplines.

The reorganization is the result of discussions that have been taking place within NSF since early in the summer, and are partly in response to outside criticism that NSF has not been doing enough to encourage engineering research, with the result that engineering has suffered in comparison with more traditional basic science disciplines.

At the same time, NSF has dropped a proposal that had also been under discussion to set up a new directorate for social science, at present linked with the biological and behavioural sciences. Although many researchers had argued that such a move could help to enhance the academic status of social science research, administrators had voiced fears that it might increase the visibility of the social science research budget — and hence its vulnerability to congressional budget cuts.

According to Dr Slaughter, the new reorganization means that although basic research remains the central mission of NSF, the base of support for applied research will be broadened, since all research directorates will now support applied research projects while keeping their basic research programmes more or less in being.

In addition, the reorganization would

give new emphasis to engineering research and education, Dr Slaughter told the members of the National Science Board, which is formally responsible for the activities of NSF and had previously endorsed the reorganization proposals.

NSF officials hope that, by giving engineering research a new emphasis, they will be able to head off plans to set up a National Technology Foundation, contained in a bill introduced to the House of Representatives by Mr George Brown, chairman of the House Science and Research Subcommittee.

In a letter to Dr Donald Langenberg, acting NSF director, Mr Brown said that Congress might conclude, in studying the technology foundation concept, that "existing mechanisms to improve the state of technology are not able to handle current challenges, and that major programme dislocations are not too high a price to pay for a fundamental programme reorientation".

Several members of the engineering research community, while welcoming NSF's greater emphasis on engineering, have been saying that this will only improve the situation for engineering research, which now receives only about 10 per cent of the NSF's budget, if it results in more money being made available by Congress.

David Dickson

Nuclear power

Border problems

Brussels

France's policy of siting nuclear power stations on its frontiers has aroused antagonism both in the European Parliament and among environmental groups. The European parliament met on 19 November to discuss a report by Mechthild von Alemann (German, Liberal) which reflected the concern over the French government's plans to build

further power stations at Chooz and Cattenoom, close to the Luxembourg and Belgian borders.

The previous day, environmental groups from Luxembourg, France and Belgium had called a press conference in Luxembourg and denounced the French government's total disregard for the principles and rules of international law. The French, however, are not the only culprits. Of the 120 or so nuclear power stations at present in operation, under construction or planned in the European Community, 33 are less than 25 miles from national borders and another 15 are less than 6 miles from borders.

Von Alemann's report urges the introduction of Community safety norms and the implementation of a Community consultation procedure, bolstered by an EEC regulation. During the debate many speakers called for a broader interpretation of Articles 37 and 41 of the Euratom Treaty. These relate to the obligation of member states to supply the European

Commission with all data on radioactive effluent capable of causing pollution and details of all future plans for nuclear power stations.

French parliamentarians of all political hues united against the attack, claiming that it compromised national sovereignty. Pierre Calvez (French, Liberal) said he was opposed to any Community decision which transcended national policy. He added that all new power stations were subject to bilateral agreements and international regulations.

Speaking on behalf of the European Commission, acting Energy Commissioner Etienne Davignon declared that France had not infringed the Euratom Treaty and that the treaty could not be interpreted in such a light as to give the Commission the right to interfere.

Stanley Johnson (UK, Conservative) reminded the parliament of the fate of the so-called Sèvres Directive at the last meeting of the Environment Council in June. The draft directive on the prevention of major accidents was not adopted because the French objected to the provisions for transboundary notification procedures. A compromise formula over this problem is now being thrashed out and it is hoped that the next council meeting on 12 December will adopt the text.

The EEC's energy ministers, when they met in Brussels on 27 November, briefly discussed the problems associated with the siting of nuclear power stations near boundaries. The draft regulation on the Community consultation procedure for electricity power stations that affect the territory of another member state has been held up in the council for several years. It now seems likely that this regulation will be looked at more seriously and its progress accelerated.

Jasper Becker

Soviet health

More research

A high-level cooperation programme between Soviet scientists and doctors will be an important feature of the next Five Year Plan for public health. This was decided by a joint session of the Academy of Sciences and the Academy of Medical Sciences last month. An interdepartmental scientific council is to be established, and a joint research programme is being drawn up. This says Academician Anatolii Aleksandrov, president and chairman of the new council of the Academy of Sciences, will reflect the main problems in public health and take into account the potential of the country's scientific institutes.

Aleksandrov reported that cooperation between scientists and medical teams has already led to some major advances, including medical applications of ultrasonics, cryogenics and laser technology. The Minister of Public Health, Boris Petrovskii, spoke warmly of the raised standards of diagnosis and

treatment made possible by the introduction of new techniques in cardiology, oncology, surgery, paediatrics and pharmacology.

Molecular biology, which until the mid-1970s was neglected in the Soviet Union, received a glowing tribute from Dr. Nikolai Blokhin, President of the Academy of Medical Sciences. Recent results, he said, opened up prospects for the establishment of new ideas about the nature of viruses, malignant changes in cells, human heredity and the development of genetic engineering techniques. Other innovations are less welcome. The Marxist-Leninist world outlook, said Blokhin, protected Soviet medicine from mysticism, parapsychology, the psychosomatic theories of Freud, various types of neo-Freudianism and other "views that could lead medicine astray". He called therefore for greater cooperation between medical workers and representatives of the philosophical institutes of the Academy of Sciences.

Vera Rich

Polish science

Advice not taken

The Gierek regime consistently "torpedoed or disavowed" constructive criticisms from its nine-person team of scientific advisers. So claims Professor Alojzy Melich, a member of the team since its inauguration in 1977, in an interview in *Trybuna Robotnicza*, the daily paper of Katowice (Mr Gierek's home power-base).

Professor Melich said that during the past three years, the team had prepared about a dozen comprehensive surveys on the main problems facing the country, as well as numerous smaller papers and analysis. These, however, were rejected by the government and the planning commission, simply because they attacked current policies. In the end, Professor Melich said, the government simply stopped providing its scientific advisers with any information at all.

This is not the first such accusation to be levelled in recent months. In August, an *ad hoc* farmers' committee which has since become the nucleus of the farmers' campaign for trade union representation, accused the authorities of suppressing expert reports on the deterioration of Polish agriculture. Unlike most critics, however, Professor Melich did not condemn Mr Gierek's policy of obtaining massive foreign loans to back his investment projects; these, said Melich, had provided an important stimulus for the economy.

The current economic crisis, however, has meant that the future of many major investment projects has become doubtful. Even the much hailed Programme-Wisla is liable to suffer some cutbacks at least for the present. This scheme, which was envisaged as a major contribution to Poland's economy in the twenty-first century, consists of a broad range of water-

French take Manhattan

A French firm has stormed the US optical communications market — and installed a 6.5-km optical fibre system in Manhattan. CIT-Alcatel, the leading French telecommunications company, announced last week that it had beaten off competition — including the giant ITT — to supply the link to Western Union cable and telegraph company. They system will be the first fully operational optical fibre link in the United States CIT officials believe.

CIT will also supply the second such link, an experimental one which the company sold to Western Union a year ago. When the new link, which uses light emitting diodes to convert electrical signals into light, goes into operation in January, the experimental link (which uses laser diodes) will also be put into regular use. CIT will thus gain operating experience with the two principal competing technologies for optical fibre input.

The great advantage of optical fibre systems for Manhattan is that available cable ducts under the city are physically crammed to capacity, and it is now almost impossible to feed through standard electrical links. Optical fibre links, however, can be made on cables 10 times smaller in outer diameter — allowing a hundredfold increase in channel capacity for the same duct cross-section.

The CIT system uses cables, supplied by another company, of 1 cm outer diameter to carry 44 megabits per second (672 telex channels). CIT supplied the input-output devices and the data management systems for the link. CIT considers that this first step in the US market is "fundamental" and that the potential scale of the market is "huge".

Robert Walgate

Cable of protest

One hundred and six Fellows of the Royal Society, including four Nobel Prize Winners, (Sir Nevill Mott, Sir Max Perutz, Sir George Porter, and Dr Fred Sanger) have sent the following telegram to the Soviet Delegation to the Madrid Review Conference.

"The arrest of Dr Brailovskii is a cause of great concern which can only harm further scientific exchange between our countries."

Viktor Brailovskii, arrested on 13 November, the day after the opening of the Madrid Conference, is thought to be facing charges under Article 191/1 of the Soviet Penal Code: Disseminating information known to be false and detrimental to the Soviet Union and socialist system. His detention, however, seems to be linked with his activity as host to the Sunday seminars for Jewish refusenik scientists in Moscow. Last week, his wife was warned by the security authorities not to try to go ahead with the seminar. When, on Sunday, she attempted to hold the seminar, intending participants were prevented by the police from entering the apartment.

In Washington last Sunday, the Committee of Concerned Scientists organized a seminar on the style of the Moscow seminar. A paper was read by Dr Maxine Singer who earlier this year was refused a visa to visit Moscow, where she had intended to visit the Brailovskii seminar. Several of the participants had read papers at the Brailovskii seminar during professional visits to Moscow. It is hoped to hold such seminars every Sunday in various US academic centres.

An international delegation of scientists, led by the French Nobel Laureate Andre Lwoff, who is a corresponding member of the Soviet Academy of Medical Sciences, is seeking a meeting with Ilichev, head of the Soviet delegation in Madrid, to discuss the Brailovskii case.

Vera Rich

managed developments, ranging from recreation to heavy transport, with canal links through the Soviet Union and East Germany. Such a major project cannot, of course, be simply abandoned, but as the press spokesman for the programme recently pointed out, it is a complex plan, the implementation of one part does not necessarily depend on that of another, and, in any case, the announced completion deadline of the year 2000 had rather "symbolic" significance. Nevertheless, he said, the country simply cannot afford *not* to implement some parts of the scheme as soon as possible — the anti-pollution and water-storage proposals which will be vital in the next few years if the country's agriculture and industry are to recover.

A similar stance was adopted recently in the Sejm (Parliament) by Deputy Zbigniew Kledecki. Speaking on the pollution of the

Krakow area by the Nowa Huta steel mills and the Skawina aluminium plant, he stated that the cost of installing modern, non-polluting equipment would be 120 million zloty (£2 million) at Nowa Huta and 7,500 million zloty (£125 million) at Skawina. Nevertheless, he said, in view of the grave pollution hazards and the "critical attitude of community scientists and journalists in Krakow", preparatory work on modernizing the plants would begin next year.

Important as such major investments still may be, the most immediate investment problem is that of agriculture. Recent pledges by the Minister of Agriculture, Leon Klonica, include an increase in the production of plant protection chemicals, and fertilizers, investments in land-improvement, development of an intermediate technology suitable for small farms, changes in the supply and pricing system to aid the private farmer, and a relaxation of central arbitrary planning in agriculture. These last points, which are a considerable reversal of recent policy, have been welcomed by the farmers with cautious approval.

Vera Rich

Brazilian universities

No cash ahead

Sao Paulo, November

Inflation and a lack of sympathy from the state government have forced the University of São Paulo (USP), Brazil's oldest university and one of its best, into a serious crisis. But the crisis is not confined to São Paulo. All government-supported universities face difficulties over dwindling budgets. Academics throughout Brazil have been protesting about the lack of resources by holding a series of one or two-day stoppages, the latest at the beginning of this month.

The academics' complaint is that the state and federal governments have made the universities take an unfair share of public expenditure cuts and have thus neglected the country's long-term future in the panic to service Brazil's massive foreign debt and oil import bill. This results in the universities having virtually no money for new building and very little for buying and maintaining equipment.

The most serious complaint, however, is the effect of inflation on academic salaries, which have recently been increasing at only about half the rate of inflation and which are corrected only once a year. (The academics are asking for termly readjustment.) The lack of money available for salaries has meant that some academics have to turn to part-time and piecemeal teaching. Many academics can get contracts for only 20 hours a week and have to eke out a living by other means. But even those with 40-hour contracts are finding it necessary to look for ways of topping up their incomes.

Salary problems seem to vary from one

university to another and depend on how individual funds are administered. Some federal universities, called *fundações*, are allowed to keep their own trust funds which are being used to top up salaries, but others, the *autarquias*, are strictly limited to paying agreed government salaries only. For those in the humanities in the *autarquias*, the outlook is bleak, but for scientists there is a chance of extra support from the national research councils, which have recently started to top up researchers' salaries as well as making research grants. The budgets of the research councils have not, however, stretched to helping every scientist worthy of support.

The number of applications made to the main research council, the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for grants to supplement salaries increased dramatically in 1980. The CNPq estimates that it is now supplementing about 2,200 scientists' salaries and that its sister organizations may be supplementing as many as 3,000. Most notable, says the CNPq, is the large increase in applications from the University of São Paulo.

For federal universities, the CNPq is confident that the problem is only temporary and that the salaries problem will be alleviated by the beginning of the next academic year in March. The University of São Paulo, however, cannot necessarily expect such a reprieve. Its finances are controlled by the government of the state of São Paulo which allocates the budget annually — the most serious deterioration in the university's finances has occurred over the past few years, since a change in the state government.

If São Paulo has been hit more severely than most of the federal universities, it may be — according to one administrator — that the university had more to lose. But many academics and newspaper reports blame the crisis on the governor of São Paulo state, Paulo Maluf, who is keener on oil exploration projects than on the university.

The university's teachers' association says that its share of the state budget decreased from 3 per cent in 1975 to 1.89 per cent in 1980, and that the share taken by higher education generally decreased from 5.1 per cent to 3.3 per cent over the same period. The same figures put the average salary of a full-time professor at about 80,000 cruzeiros a month (£6,000 a year), less than half of its maximum real value in the past decade. But budgets are not the only problem afflicting Brazilian universities. Their rapid expansion from 500,000 students in 1968 to 1.5 million now has led to unwieldy bureaucracies which changes of government have only aggravated. At São Paulo, the seat of university unrest in 1968, academics say that even relatively small items of expenditure have to be approved by the rector, who in turn has to argue for funds with the state government.

Judy Redfearn

High-energy physics

Machines shut down

A rise in electricity prices of nearly 50 per cent has forced the West Germany high-energy physics laboratory, DESY in Hamburg, to close down its accelerators until the end of 1980. The federal government in Bonn has asked the regional Hamburg government to foot the bill, but Hamburg refused — so DESY had to shut, losing three weeks of accelerator time.

Electricity costs have risen because of a recent agreement between the utilities, coal mining companies and the government. Under the agreement, coal used in electricity production will be raised from its present 33 million tonnes a year to 45 million tonnes by 1985. The utilities will thus have to invest in new power stations and the federal government has allowed them to raise the necessary cash from the consumer.

DESY will have to find another DM 5–10 million (£1–2 million) in 1981 to cover the increased electricity charges. If the money is not forthcoming, the accelerators will have to be run at two-thirds mean power. This would mean either low-energy experiments or relatively few runs at high energy. For example, Nobel laureate Samuel Ting's experiment to find interference between the weak and electromagnetic interactions, as evidence for the intermediate vector boson, requires the highest energies and long data runs and may be threatened unless one or other government pays the increased bill.

The federal government in Bonn, however, which through the Bundesministerium für Forschung und Technologie (BMFT) provides 90 per cent of DESY's budget, is in the red, and has been asking all public institutions to find savings. DESY's DM 143 million budget for 1980 has already suffered two cuts — DM 10.5 million in its DM 50 million "capital projects" budget (achieved by delays) and DM 1.5 million in running costs. BMFT also seems set not to grow this year, its provisional increase for 1981 being slightly less than the current 5.3 per cent annual growth in the cost of living in Germany.

In principle, the Hamburg government could increase its 10 per cent contribution to DESY, as it already benefits from DESY's policy of placing many of its equipment contracts with local industry, and it owns the electricity company which is now charging the laboratory so highly. So far, however, it has not done so.

The DESY crisis may prove to be a test of the new science minister's faith in basic science. Dr von Bülow announced at a press conference last week that he was in favour of "full government financing" for basic research, whereas in energy research, for example, industry must make a contribution.

In the long run, physicists have pinned DESY's future on the construction of

HERA, a DM 600 million machine to collide electrons with protons at high energy and so probe proton and quark structure; the project is being weighed against other big science projects by a committee of BMFT, and no doubt electricity costs will be taken into account. DESY's electricity bill this year will be about DM 22 million, with the full HERA, at current prices, about DM 43 million. So DESY is making a great effort to develop superconducting bending magnets for the proton ring to keep down the bill. It has two designs, one based on the successful dipole constructed at Fermilab in the United States and another under construction at Saclay in France.

Moreover, it is still planned to build HERA in two stages, the first involving 35 GeV electrons and positrons alone and costing DM 290 million. This stage could be ready 5½ years after approval (at the end of 1986 at the earliest) and still offers some competition for the large electron-positron ring (LEP), the next major project of the European nuclear research centre CERN at Geneva. LEP's electricity costs for the same energy would, however, be lower as its ring would be much larger, and it would radiate less synchrotron radiation (the major energy sink in a circular electron machine).

Robert Walgate

Third World

Consumers arise

Kuala Lumpur, November

At a time when the new Administration in the United States seems likely to ease up on the stringent regulation of technological products, consumer groups in the Third World are beginning to voice demands for protection against potentially harmful substances and goods like that already adopted by more affluent societies.

A principal target of such groups in the alleged "dumping" in developing countries of products, such as drugs and pesticides, which have been banned in one or more of the industrialized nations.

Three weeks ago, for example, individuals from twelve developing nations attending a conference in Malaya organized by the International Organization of Consumer Unions (IOCU) signed a declaration urging that there should be no distinction between domestic and foreign consumers in export control programmes for hazardous substances and production facilities.

But the concerns of the embryonic consumer groups in the Third World go much further, ranging from the conventional complaints about the quality of consumer goods to criticism of low-standard imitations of Western goods (for example a drug named "Panadoi", easily confused with "Panadol").

Malaya's Finance Minister, Tangku Razaleigh Hamzah, has called on university scientists and other faculty

members to put their efforts behind the work of such consumer associations, which should be "watchdogs of the people" surveying the unfair practices of businessmen.

Tengku Razaleigh was speaking at a seminar on economics and development organized by one of the largest and most active of Third World consumer groups, the Consumer Association of Penang (CAP).

Formed more than ten years ago on the Malayan island of Penang, CAP now has a full-time staff of 60. Its activities have covered a range of issues familiar to pressure groups in industrialized countries, from assisting fishermen whose livelihood was threatened by the discharge of toxic wastes from a new factory, through working with local labour unions to identify occupational carcinogens, to developing courses and teaching material on consumer rights for use in schools and universities.

One of the successes claimed by CAP was the Malay government's decision to accept explicit responsibility for environmental issues by expanding the portfolio of the Ministry of Science and Technology. Various controls on pollution sources have since been introduced, but less progress has been made on efforts to curb the rapid exploitation of Malaya's diminishing natural resources. As well as tin and petroleum, these include hardwood forests which may disappear completely in ten to twelve years if trees continue to be cut down at the present rate.

Taking a lead from CAP, and in line with widespread concern among the developed nations, many universities have begun courses in environmental sciences in the past decade in Malaya. The University of Malaysia in Kuala Lumpur has recently introduced a course in consumer law as part of its law degree, taught by one of CAP's founders in Penang.

But both CAP and IOCU — whose current president, Mr Anwar Fazal, was another CAP founder — argue that Western concepts of environmental and consumer protection should be expanded to include concern for all aspects of industrial production, from factory working conditions to community health.

Even though Malaya has in recent years passed a variety of laws covering all such areas, CAP claims that government authorities are frequently slow or reluctant to put these laws into effect. Officials reply that the magnitude of the task that they face, and the continued pressures for economic growth, make it difficult to move any faster.

In a country where opportunities for political dissent remain limited — CAP recently moved its Asian headquarters from Singapore to Malaysia — CAP officials tread warily on the boundaries of legitimacy, aware that their required registration as a society could be questioned if their activities become too overtly political.

But given the relative weakness of labour unions and official opposition parties, the consumer movement has become one of the few direct routes for attempting to influence government policies. "We create pressures within the bureaucracy" says Martin Khor Khok Peng, CAP's research director. "If we educate the public, the bureaucrats will have to listen."

David Dickson

Conservation

Bill of rights

The British government is hoping that its new Wildlife and Countryside Bill, introduced in the House of Lords last week, will be law by the end of next summer. Its passage through parliament, however, may not be easy. The bill has taken a long time to compile, preliminary consultation papers having aroused considerable opposition from conservation groups which do not feel that all inadequacies have been ironed out in the latest draft.

The chief effect of the bill is to bring British law in line with that of Europe, but Mr Tom King, minister for local government and environmental services, stresses that many of the new measures are badly needed anyway. The clauses in the bill on the protection of birds, methods of killing wild animals and the introduction of exotic species fulfil European requirements while those relating to nature conservation, the countryside and national parks are designed to improve wildlife management.

Mr King is especially pleased with the clauses relating to the management of areas designated as sites of special scientific interest (SSSIs). The conservation groups, however, say that these clauses are amongst the most worrying in the bill. Under them,

owners of some SSSIs will have a statutory obligation to inform the Nature Conservancy Council (NCC) of any planned operations which may affect the physical or biological features of the site and to wait for up to a year for the consent of NCC. Failure to notify would carry a penalty of up to two years imprisonment and a fine of £1,000 or both.

The conservationists will be seeking to amend these clauses to include all SSSIs, not just those singled out for special treatment, and to include some statutory obligation on the landowner to carry out the management recommendations of NCC. Under the bill, if agreement on management of a site is not reached within one year of notification, the landowner is free to carry out his original plan, although he may risk compulsory purchase by NCC.

On the whole, conservationists welcome clauses in the bill relating to the protection of wild birds, animals and plants, seeing them as a major improvement over existing legislation. The bill will tighten up the laws on the import and export of endangered species, methods of killing wild animals, keeping birds in captivity and selling them either dead or alive. Most of these amendments are in line with the European Community Directive on Wild Birds. The bill also seeks to protect "rare" creatures, such as the otter in Scotland, before they reach the endangered list.

A serious omission in these clauses, however, say the conservationists, is the lack of adequate provision for enforcement. They will be lobbying for the creation of a small investigation unit, probably under the aegis of the NCC, to give support and expert advice to customs officials and police who often miss infringements in the law because of unfamiliarity with wildlife.

Recent controversies about the main-

Fallout from China

China's latest nuclear test on 16 October was "dirty" — according to the official Polish news agency PAP. The dust cloud had previously been monitored in Japan and New York, but the Polish agency reports of work from the Central Laboratory of Radiological Protection outside Warsaw is an example of the new policy of openness adopted by the Polish press. The laboratory has been working in the field since about 1973 with little publicity in Poland.

The Polish team, in the person of its leader, Dr Zbigniew Jaworowski, says that it has observed a significant amount of radioactivity at a height of 15 km. The Polish monitoring method is novel. A converted Mig-18 fighter flies horizontally through the dust cloud, opening a collecting duct at a predetermined altitude. The results suggested a fission-fusion-fission device, and this information was made available to the American group at the Environmental Protection Agency in Washington.

These aerial activities are not Dr Jaworowski's main interest, however. He is principally concerned with monitoring background levels of radioactivity — both natural and that produced by discharge of radioactivity into the atmosphere by "conventional" means — in Poland, largely from the combustion of coal. In September of this year Dr Jaworowski was appointed head of a United Nations commission on fallout, directly responsible to the General Assembly. His measurements of radioactivity levels in glaciers and icecaps throughout the world have met with some controversy, as he says that apart from a few black spots, the global build-up of radioactive fallout over the last century is far less than is claimed by the powerful "doomwatch lobby".

Vera Rich

English floodmeadows: threatened by ditch or protected by bill?



Photo: RSPB

tenance and safety of public footpaths are only partly dealt with in the bill. Although the rights of farmers to keep bulls in fields crossed by public footpaths will be restricted to some extent, beef bulls will be allowed if they are accompanied by cows. Ramblers will be no happier with that provision than with the transfer of general powers over footpaths from central to local government. Ramblers fear that local bias will mean that many paths are lost.

Although many conservationists agree that the bill improves on existing legislation, they consider it far from ideal. The opportunity to revise wildlife and conservation legislation comes up only about every five years so some hard fighting seems inevitable. The bill may have to take more blows than the government seems to have anticipated.

Judy Redfearn

CORRESPONDENCE

Museum pieces

SIR — Halstead's progress report on the death of scholarship and the rise of Marxism in the Natural History Museum (*Nature* 20 November¹) mixes too many different issues for a full reply. I will concentrate on his attack on scholarship in this institution. To Halstead, the symptom of decay is advocacy, in the public galleries, of cladistics, a method which he believes is being forced down the public throat by a Public Services Department which has overridden the views of scientists in the Museum.

Cladistics, as presented in the "Dinosaur" and "Fossil man" exhibits, may indeed seem oversimplified to some, or not fully thought through to others. But Halstead's alternative approach, as detailed in his comments on fossil man, seem to me far more grievously mistaken. In accusing us of lack of scholarship, he offers instead "the well attested sequence of human fossils representing samples of succeeding populations has . . . been taken as a classic example of the gradual evolution of a single gene pool. Certainly there is not any serious doubt about *Homo erectus* being directly ancestral to *Homo sapiens*". Confronted with these statements, one must either bow to Halstead's scholarship, or ask "attested" by whom? "been taken" by whom? "not any serious doubt" by whom? Halstead's answer might be, to quote the Museum handbook to the old exhibit on fossil man which was removed to make way for the dinosaurs, "the evolution of Man has come to be regarded as fact rather than hypothesis by all persons qualified to judge the evidence"². In other words, we (scientists, experts, authorities) tell you it is so.

The radical departure in the exhibit reviled by Halstead is that the voice of authority is less strident. The visitor is encouraged to understand, and to take part in, the reasoning that underpins the story of human evolution; to become one of those "persons qualified to judge the evidence". And cladistics is the logical entry to that reasoning.

Amongst scientists in the Museum there are many different viewpoints on the value and generality of cladistic methods. Those viewpoints are a symptom of activity and debate. Halstead opts out of the debate, deferring instead to the authority of Mayr and Simpson, of books published 30 or 40 years ago, "and indeed of Charles Darwin himself". From his "reading of the literature of cladistics" Halstead concludes that cladists form the opposition "to the concept of gradualism and to the idea that the processes that can be observed at the present day, when extrapolated into the past, are sufficient to explain changes observed in the fossil record". This last idea, of extrapolating the present into the past as sufficient explanation, is usually attributed to Charles Lyell under the name uniformitarianism. Lyell's uniformitarian explanation of the changes observed in the fossil record was piecemeal extinction and creation³, an explanation invoking "qualitative leaps". Indeed, T.H. Huxley himself was unable fully to accept Darwin's gradualism, and preferred the saltationist camp. In short, Halstead's equation of cladistics and saltation is simply mistaken.

Recent advocates of saltation, and critics of extrapolation from population genetics to macroevolution, such as Gould⁴ and Stanley⁵, are not cladists. Whether they are Marxists is another matter, in my view an irrelevant one.

More relevant is Halstead's confusion over the relation between cladistics, a method of systematics, and questions of process — modes of speciation or transformation of species. He sees a necessary connection between cladistics and one view of the evolutionary process, but as cladistic literature makes plain⁶⁻⁸, there is no such connection. Cladistics is not about evolution, but about the pattern of character distribution in organisms, or the recognition and characterization of groups. Halstead might direct his search for Marxist propaganda towards the Nuffield Biology texts and guides, for the teacher is urged "to develop a common vocabulary (and possibly notation) in biology and mathematics" by teaching the elements of systematics through Venn diagrams, which are logically synonymous with cladograms⁸. All that seems to lie behind Halstead's complaint is his failure to grasp the distinction between pattern (systematics) and process (explanation of the pattern)⁹. Hence his mistaken belief that he knows how evolution works, and that we are unwittingly committed to Marxist historicism.

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SIR — In Dr Halstead's pleas for traditionalism in the Natural History Museum's exhibitions (*Nature* 20 November, p.208) he makes an assumption that lends much potential support to those whose policies he is so "fervently dedicated" to trying to discredit.

It is true that Marxists have attempted to equate the idea of punctuated equilibria (in its many guises) with the principles of dialectic, the head-on collision between changed environment and less well adapted organisms giving rise, by dialectic confrontation, to the new 'adapted' order, exactly as Marx envisaged the clash of the bourgeoisie and the proletariat, though Marx himself was somewhat vague in this area. Gradualism has been called on as evidence for other political persuasions. Politicians of all colours of the political spectrum have found things in Darwinism to support their political positions. As Shaw says of Darwin, "He had the luck to please everybody who had an axe to grind".

Unfortunately Dr Halstead is himself supporting the tenuous link between change in historical and present day human societies; between so-called "cultural

evolution" (which is a true Lamarckian system in any case) and the far slower and partially understood process of biological evolution, he is approving the idea that supposed historical events in evolution can be used as "scientific" evidence to predict and explain cultural evolution. Dr Halstead has only got under the surface of the argument, not to its core; he would surely be far better off questioning this primary assumption, rather than unintentionally lending support to biologically naive political thinkers. If he could do this, the problem he feels so strongly about would evaporate and he could escape. As it is, he is in great danger of being trapped by his position; his gradualist evidence is more tenuous than he implies, and since the recent Chicago conference it is probable that punctuated equilibria will indeed become a new orthodoxy. How will Dr Halstead free himself? With a "mighty leap" — a method some orthodox neo-Darwinians have been known to use?

The confirmation of his superficial attitude is given by his statement that, "Marxism will be able to call upon the scientific laws of history in its support". Dr Halstead clearly believes, as, of course, Marx did, that such "scientific laws of history" exist, but their emptiness has been elegantly demonstrated by Sir Karl Popper in *The Poverty of Historicism*, and *The Open Society and its Enemies*.

Having read Dr Halstead's views on Popper elsewhere I am not surprised at all by his confusion.

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SIR — L.B. Halstead's letter (*Nature* 20 November, p.208) is misguided and, if his arguments were generally accepted, dangerous to the unfettered development of science. Whatever the scientific merits and demerits of the "cladist" theories behind the Natural History Museum's "Dinosaur" and "Fossil man" exhibits, it is most certainly wrong to attack them on the grounds that they might provide support for "a fundamentally Marxist view of the history of life". Indeed, it is ironic that Halstead should quote J.V. Stalin, for it was precisely his policy of encouraging the application of political criteria, rather than scientific ones, to assess scientific theories that ruined so much Soviet science for a generation.

It is really quite silly of Halstead to argue that because Marxist philosophers believe in revolutionary leaps (and, incidentally, so do several other, and quite different, philosophical and sociological schools of thought) then we ought to reject any scientific theory that explains changes in specific phenomena by way of "an abrupt leap from one state to another". How far is Halstead prepared to go with this "critique"? Should cosmologists now be called upon to reject the "big bang theory", topologists "catastrophe theory" or historians of science "paradigm breaks" because they might be construed as support for a law of Marxist dialectics?

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NEWS AND VIEWS

Open debate

From M.G. Edmunds

THE simplest questions are often the hardest. It might be thought that 'What is most of the mass in the Universe?' should have been answered long ago, but a conclusive statement still eludes us. It is not too difficult to determine the radiation output of the galaxies, stars and interstellar material that we can actually see — with 'seeing' including radio, X-rays, infrared or ultraviolet, as well as visible light. From the amount of radiation we can infer the amount of radiating mass present. The resultant mass density of the Universe is comparatively modest, being only about one-fortieth of the 'closure' density required to exert sufficient gravitational pull to eventually halt the Hubble expansion and cause a re-collapse. The consequences of a closed or open Universe are rather remote on human timescales, but the problem exerts a strong philosophical attraction. Hence it is of considerable interest that there is growing evidence for a large amount of mass which does not radiate enough to have been seen, yet which might go a long way to making up the density required for closure.

The idea of 'missing mass' — mass that is present but not detected apart from its gravitational influence — is not a new one, and dates back to investigations of the dynamics of clusters of galaxies in the 1930s. In giant clusters, the rapid motions of the galaxies imply the existence of a gravitational potential well that is much deeper than can be explained by adding up the assumed masses of the visible galaxies. For many years there existed a feeling among astronomers that the 'missing mass' problem would eventually just go away when better observational data, or more appropriate theoretical treatment of that data, became available. But the problem has not gone away, and improved observations have all pointed to an upward revision of the mass density.

The most persuasive evidence for non-luminous matter comes from observations of the rotational velocities of spiral galaxies out to quite large distances from their centres (see, for example, Rubin, Ford & Thonnard *Ap.J.* **238**, 471; 1980). In many cases the velocities do not fall off with

distance from the nucleus but stay constant. A little elementary physics indicates this implies that the total mass inside a given radius increases linearly with radius. Yet the light output decreases exponentially with distance from the centre, so the matter in the outer parts must be many times less luminous per unit mass than the material in the inner parts. Since there is no evidence for a large amount of hidden material in the disk of our own Galaxy, it is likely that the dark mass in spirals has an extended, or 'halo', structure. Again from our own Galaxy, there is no evidence that the mass is in the form of faint stars.

It is obviously important to know how far the dark haloes extend, and how much they contribute to the total mass of a galaxy. Some evidence comes from the dynamics of small groups and binary pairs of galaxies, although the interpretation is difficult (see, for example, the excellent review of the whole question of galaxy masses by Faber & Gallagher *Ann. Rev. Astron. Astrophys.* **17**, 135; 1979; although considerably more galaxy masses have been published since this review, the general picture remains the same). Widely separated galaxies should sample the whole of each others' gravitational field, and such evidence as there is suggests the haloes could extend to 100 kpc, which is five or ten times the nominal visible extent of a typical galaxy. The dark material contributes as much, and probably several times as much, mass as the visible material.

If galaxies are considerably more massive than previously supposed, then this goes some way towards explaining the high dynamical mass implied for giant clusters of galaxies — but probably not all the way. There may still be several times more mass in the cluster than can be explained by adding up the (now heavier) individual galaxies. A new method of illustrating the presence of hidden material on an even larger size scale has been recently proposed (Davis, Tonry, Huchra & Latham *Ap. J. Lett.* **238**, L113; 1980). M.G. Edmunds is in the Department of Applied Mathematics and Astronomy, University College, Cardiff.

which uses the motion of our local group of galaxies against the reference frame of the cosmic microwave background radiation. When this motion was first detected its direction was something of a surprise since it did not coincide with previous determination of the local group motion relative to other galaxies. Although the differences between the two motions are not fully understood, Davis *et al.* suggest that the component of motion towards the Virgo supercluster of galaxies, as determined in the microwave data, can be used to estimate the mean mass density of the Universe. They do this by considering the peculiar motions induced by perturbations of density (such as the Virgo superclustering) on an expanding Hubble flow. Their derived mean density is as high as four-tenths of the critical density required for closure. Thus the relative amount of mass which is hidden from view appears to increase with the linear size of the system — it is barely noticeable in the inner regions of galaxies, but becomes apparent at the outsides, it markedly influences groups and pairs, then dominates clusters and superclusters.

So what form is the dark mass in? The question is now a very important one since the dark mass is considerably greater than the visible mass. A population of low-mass objects can be imagined — interstellar material which condensed, became electron degenerate and is able to support itself without nuclear reaction. Such objects would have to be individually less than about one-twentieth of a solar mass. Large numbers of black holes or neutron stars are another possibility. But, apart from the difficulty of inventing a coherent picture to account for the formation of such objects without violating other constraints (notably limits to the amount of synthesis of heavy elements), there remains a fundamental problem if the missing mass is to be in conventional nucleon form. The dilemma comes from the effect of the extra mass on the nuclear reactions which synthesized helium and deuterium in the Big-Bang fireball at the beginning of the Hubble expansion. Putting the missing mass into nucleons

causes increased synthesis of helium and decreased synthesis of deuterium (see, for example, Yang, Schramm, Steigman & Rood *Ap.J.* **227**, 697; 1979). It might be possible to account for the observed deuterium in the Universe by production in other sites — perhaps supernova envelopes, although nobody has been able to think up an efficient enough mechanism. But overproduction of helium is a much more difficult problem, since it is not significantly destroyed (but rather added to) in normal stellar evolution and the observational limits on the maximum amount which could have been produced in the Big-Bang are now quite good.

The nuclear arguments would imply that the mass of nucleons in the Universe is not more than a tenth, and probably not more than a twentieth, of that required for closure. This corresponds to what we see in galaxies, although the error bars might just allow us to squeeze in the extra mass in galactic dark haloes as nucleons. The hidden mass in clusters, or that implied by Davis *et al.*'s Virgo supercluster argument would cause too much primordial helium production and far too little deuterium. A possible solution lies in the existence of a sea of massive neutrinos, left over from the Big Bang. The case for such a sea has been eloquently argued by D.N. Schramm and G. Steigman in this year's Gravity Research Foundation first-prize essay (to be published in *General Relativity and Gravitation*. See also Sybailis, Yang & Schramm *Nature* **288**, 143; 1980). They argue, on the basis of consideration of the clustering properties of massive neutrinos (see Tremaine & Gunn *Phys. Rev. Lett.* **42**,

407; 1979) that the most likely neutrino mass would be $3\text{eV} \leq m \leq 10\text{eV}$. The extra mass of these neutrinos would allow the missing mass in galaxy clusters to be explained, but problems would still remain if Davis *et al.*'s (albeit indirect, and somewhat uncertain) mass density is correct, or if closure is desired. The difficulty is that putting more and more neutrinos into the Universe will eventually cause degeneracy of the neutrino sea, which would again affect primordial element production. At one-tenth closure density, this does not appear to be a problem, but at much higher densities a more massive neutrino would be required, and it is not clear that such a neutrino would cluster in the right way to provide the increase of missing mass fraction with linear scale of the system considered. Astrophysicists will be eagerly awaiting experimental evidence of neutrino masses (see *Nature* **286**, 755; 1980), and further investigation of the clustering of neutrinos would seem important.

Whether the majority of mass in the Universe really is a sea of neutrinos, or whether it is conventional nucleon matter, remains to be decided. But the existence of considerable dark matter has been demonstrated. It is not so long ago that a headline appeared in a North American newspaper reporting "Caltech astronomers declare Universe open". Although the evidence still remains in favour of openness, one cannot help wondering if (in apparent obedience to current financial stringencies) the Universe may not be all that far from closing again. □

expanded the structural details along more speculative lines to propose evolutionary trees based on the variations between polypeptide subunits in ATPases.

The argument still rages in mitochondrial bioenergetics as to how many protons are translocated at each energy conservation site in the electron transport chain, S. Papa (Bari) favouring two whilst other groups favour three or four. Unfortunately, experimental measurements show little prospect of resolving this problem. Since extensive research has failed to implicate any quinone in the transfer of electrons from cytochrome *b* to cytochrome *a*, there is speculation about an alternative mechanism of proton translocation, possibly involving multiple H^+/e ratios. In contrast, research using chromatophores has provided strong evidence for the involvement of a specific quinone (Z) between cytochromes *b* and *c*, and the requirement for a quinone pool for ATP synthesis.

New evidence for the role of quinones in electron gating was discussed by A. R. Crofts (Urbana). In both chloroplasts and photosynthetic bacteria there is evidence that a quinone reduces cytochrome *b* only on even flashes of light, that is, only after the formation of QH_2 , which would fit with the original ideas of a Q cycle. As Z has been identified as different from Q_2 however, this must be rejected and an alternative mechanism of quinone oxidation invoked, although the proponents of a linear chain model have not explained how it copes with two electrons simultaneously. The concept of gating raises many problems, not least being the interpretation of the results of repetitive flash experiments used to measure rates of electron transport in photosynthetic systems. In mitochondria, although much evidence now points away from a classical Q cycle, no real alternative was put forward.

A system for examining an alternative non-Mitchellian mechanism of proton translocation is the bacteriorhodopsin proton pump from halobacteria. However, it was evident from the discussion that this apparently simple system is resistant to experimental analysis and is still a long way from providing a useful model. One recent, interesting development is the discovery that there is also a light-driven sodium pump in these organisms, possibly using a second retinal protein (J.K. Lanyi, Max-Planck-Institute, Munich).

Calcium transport was covered extensively but no new theories were presented and the sessions were devoted to discussing the alternative mechanisms for calcium release from mitochondria which are sodium insensitive. There is still no consensus as to which of three different methods is the more probable, polyunsaturated fatty acid induced-efflux, oxidation of mitochondrial pyridine nucleotides or an indirect inorganic phosphate-induced pathway.

Bioenergetics in Europe

from Judith Armitage

THE First European Bioenergetics Conference, held in July in Italy, showed that, with the general acceptance of the chemiosmotic concept of energy transduction, interest has moved from the gross theory to the finer, molecular and genetic details.

The problem of elucidating the functional, *in vivo* structure of the membrane-bound energy-transducing proteins using isolated systems and physical techniques was one of the basic themes of the conference. This problem was well illustrated by the many discussions on the possible structure of cytochrome oxidase, and its relation to function. R. Capaldi (Oregon) using X-ray diffraction and image reconstruction on the isolated enzyme described the possible functional

arrangement of the seven polypeptides across the membrane. Although local interactions *in vivo* and the precise role of each subunit is still unknown, the experimental data and *in vitro* structural studies were used by M. Wikström (Helsinki) to speculate on the possible mechanism of proton translocation by the protein and its relationship to the conservation of the membrane potential, emphasizing the need to consider both molecular and electrical topography within the protein.

Using very similar *in vitro* techniques on the crystallized $\text{F}_1\text{-ATPase}$ M. Amzel and coworkers (Johns Hopkins) proposed a model for the structure of $\text{F}_1\text{-ATPase}$ as a functional dimer, rather than the previous triple/single structure. There are, however, at least two arrangements of the subunits which would yield a dimer and fit *in vivo* experimental data. Some speakers

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100 years ago

The recently-presented budget of Prussia shows that, despite the financial straits of the kingdom, no considerations of economy are allowed to hamper the growth of its scientific and educational system. First on the list come the nine universities with an allotment of 7,050,000 marks (352,500*l.*). Berlin receives the lion's share, 1,378,348 marks, an increase of about 37,000 marks on its last annual subvention. Bonn and Königsberg each have 740,000 marks, Breslau 600,000, Kiel 404,000, Marburg and Halle each 430,000, Göttingen 201,000, and Greifswald 136,000. Of the above-mentioned sum about 1,306,000 marks are appropriated for extraordinary expenses in connection with the construction of university

buildings, and of this amount Berlin absorbs over one-half, viz., 766,000 marks. The other chief items in the Budget of Public Instruction are: Gymnasias and Realschulen, 5,000,000 marks; primary schools, 14,500,000; orphanages, schools for the blind, deaf and dumb, &c., 300,000; technical schools, and for the general furtherance of science and art, 3,000,000 marks.

The number of pupils of Lycees and Colleges in the French Republic is 87,000 (46,500 for Lycées and 40,500 for Colleges). Last year it was only 84,700. These establishments may be considered as analogous to the English grammar-schools.

Mr. Mundella has been speaking on education again, repeating essentially the old story, that our country must lose in the race unless, as in other countries, education in science is made an imperative part of elementary education. We have many natural and traditional advantages over other countries, but all these must in the long run succumb to scientific training.

From *Nature* 22, 2 December, 106 & 115, 1880.

Historically, Heuser had been interested in the use of rapid freezing to study exocytosis in neurotransmission. To trap exocytosis during membrane fusion and allow examination by freeze fracture techniques, Heuser and his colleagues devised an apparatus for the very rapid freezing of nerve-muscle preparations in liquid helium a few milliseconds after electrical stimulation (*J. Cell Biol.* 81, 275 1979). From here it was a small step to apply this rapid freezing, platinum replica technique to other preparations such as fibroblast cytoskeletons.

What advantages does this approach have? Heuser suggests that it avoids the use of chemical fixation of whole cells, a procedure which, by definition, is denaturing and which may, therefore, be a source of artefacts. Heuser and Kirschner compare the images of triton cytoskeletons that were prepared before or after fixation with glutaraldehyde. The filaments in cytoskeletons prepared from fixed cells appeared with variable thickness, partially agglutinated and covered with irregular deposits while those prepared from unfixed cells were more discrete entities, had more regular and consistent dimensions, and were frequently recognizable, for example, as actin filaments or intermediate filaments. The general appearance of the cytoskeletons prepared from fixed cells resembled the 'microtrabeculae' described by Wolosowick and Porter (*Am. J. Anat.* 147, 303; 1976; *J. Cell Biol.* 82, 114; 1979), who have described this structural matrix in whole cells fixed with glutaraldehyde and viewed by high voltage electron microscopy. The similarity of the cytoskeletal preparations from fixed cells has led Heuser and Kirschner not to question the existence of the microtrabecular lattice but to suggest that its appearance, with irregular dimensions and anastomosing filaments may result from the use of glutaraldehyde and the consequent cross-linking of filaments and

A new view inside cells

from Keith BurrIDGE

LIKE so many areas of science, electron microscopy does not advance smoothly but in quantal jumps which are often the result of the introduction of a new technique. The use, for example, of glutaraldehyde as a fixative by Sabatini and his colleagues in 1963 made possible the visualization of the cytoplasmic filaments that are so much studied today.

Perhaps the most recent and exciting advance in electron microscopy, gracing the pages of the *Journal of Cell Biology* with dramatic images, has come from J. Heuser and his colleagues (*J. Cell Biol.* 84, 560; 1980; Heuser & Kirschner *J. Cell Biol.* 86, 212, 1980). Heuser's approach differs

greatly from the conventional method of examining thin sections of cells that have been fixed (with agents such as glutaraldehyde and osmium tetroxide), dehydrated, embedded, sectioned and stained with heavy metals. Instead, Heuser and his colleagues have rapidly frozen either whole cells or cytoskeletal preparations (cells extracted with nonionic detergents), opened the cells by freeze fracture, removed the volatile components by freeze drying, and rotary shadowed the remaining structures with platinum. These platinum replicas are then examined by transmission electron microscopy (TEM) (Figures 1 and 2).

Figure 1 Two views of cytoskeletal elements. *a* is a tangle of filaments from the perikaryal region of a freshly plated cell which would stain diffusely for actin by light-microscope immunocytochemistry. The filaments display a 5.5nm repeat or graininess that appears to be characteristic of actin filaments as revealed by this technique. *b* shows filaments from a similar area but after decoration with the S1 fragment of myosin. (From J. Heuser and M. W. Kirschner *J. Cell Biol.* 86, 226, 1980.)

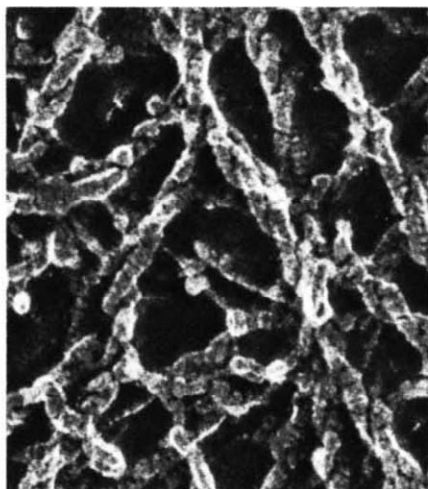
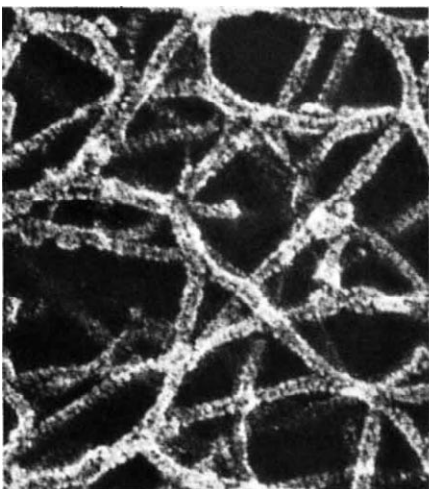
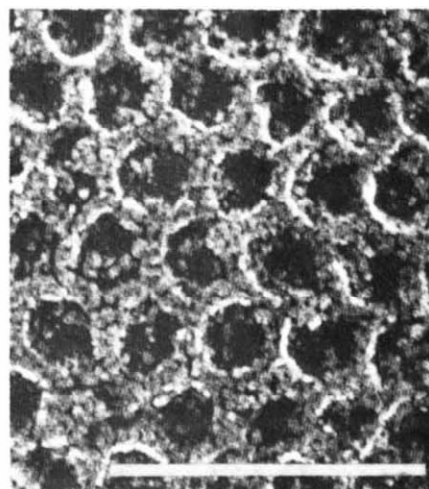


Figure 2 High magnification of a coated pit area on the cytoplasmic face of the plasma membrane of a fibroblast. Bar = μm . (From J. Heuser *J. Cell Biol.* 84, 564; 1980.)



adsorption of cytoplasmic proteins to them during fixation.

Although this may be true, it is important to realize that when Heuser and Kirschner make a cytoskeleton without fixation, many proteins are extracted and these most probably include a number that bind to or cross-link the filaments such as the actin-binding proteins (filament, α -actinin, myosin, etc.). Whereas the cellular images obtained by Wolosowick and Porter may contain fixation artefacts that contribute to the microtrabecular appearance, equally the images of Heuser and Kirschner may be oversimplified by the extraction of many of the bridging elements. Perhaps a more accurate image lies somewhere between these two.

A second advantage of Heuser's approach is that the image comes from the platinum replica. With conventional thin sections and most whole mounts viewed by TEM the final image is due to the selective binding or deposition of heavy metals, such as osmium, uranium and lead. Since the chemistry of this selective staining has not been fully worked out, there is a degree of uncertainty in the interpretation of the final image. In particular, if a cytoplasmic structure is not stained, it will not be seen. On the other hand, the replica technique should mirror the surface of the cytoplasm, cytoskeleton, or intact cell very closely. However, herein lies a problem and a limitation of the technique. When unfixed cells are rapidly frozen and then fractured so as to reveal the cytoplasm, it is difficult to reveal any structure other than a complex granularity. To obtain unimpeded views of cytoplasmic structures such as the various filaments, this granularity must be extracted or diluted. Hence cytoskeletons are excellent material for this approach but whole cells present problems. These may be partially overcome by swelling the cell briefly in distilled water or by fixing a cell first and then washing it in distilled water. Presumably, both procedures eliminate the salts and other unetchable components, but the one method reintroduces the problem of chemical fixatives whereas the other exposes cells to an osmotic shock. Although these approaches do not appear suitable for examining the cytoplasm of whole cells, they have generated some very interesting views of the cytoplasmic face of the plasma membrane (*J. Cell Biol.* **84**, 560, 1980). Indeed, the ability to view the cytoplasmic face of the membrane may be one of the most valuable contributions of this whole technique.

Part of the appeal of the images achieved by Heuser's technique comes from the depth of the three-dimensional view (similar in appearance to scanning electron micrographs). Viewing only the surface of structures avoids superimposing their upper and lower faces which occurs with thin objects in conventional thin sections.

Examining the platinum replicas of microtubules after rapid freezing and fracture, it is possible to discern the tubulin subunits in a 3-start helix, something that has only previously been seen after optically filtering electron micrographs of negatively stained preparations. Similarly, the geometry of coated pits and vesicles is particularly clear in Heuser's pictures since the upper and lower faces of these structures are not superimposed (Figure 2).

The resolution of the technique as used by Heuser is about 2nm and easily picks up, for example, the 4nm repeat of tubulin monomers in microtubules. The resolution is sufficient to distinguish the different filament types by their surface

substructure, although the resolution is less than with negative stain. When actin is decorated with the S1 subfragment of myosin, the image of an actin filament is transformed into a double-stranded rope-like structure. Like the underlying actin filament, this reveals a 5.5nm axial periodicity (Figure 1). Heuser and Kirschner also demonstrate that the technique has sufficient resolution to visualize IgG molecules directly without additional electron-dense labels. This application is particularly exciting and may permit the ultrastructural localization of accessory proteins which previously have been seen in the filament systems only by immunofluorescence.

Pathways of endocytosis

from Michael Geisow

ENDOCYTOSIS is an elegant biological solution to two related needs of living cells — the need to retrieve material bound to the plasma membrane and the need to recover the membrane itself. But what components of the membrane enter the cell and what happens to them?

Exogenous ligands — nutrients or effectors — bound by receptors at the plasma membrane collect within differentiated regions known as coated pits. The cytoplasmic coat is thought to be composed of clathrin and, provided that the temperature is above 10°C, bound ligands are cleared from the coated pits, to lodge [ultimately] in cytoplasmic structures containing hydrolytic enzymes.

The composition of coated pits is of some interest. It has been presumed that coated pits are enriched in the appropriate receptors which enter the cells together with bound ligand. Naturally, this assumes the continued fidelity of the receptor part of the labelled ligand complex. What other components of the plasma membrane also disappear into the pit?

In the fibroblastic cell at least two intrinsic membrane polypeptides appear to stay clear of coated pits. Ferritin markers linked via specific antibodies to these two membrane proteins (the θ and H63 antigens) were found by Mark Bretscher and colleagues to be excluded from pits¹. In contrast, ferritin and other labels conjugated to low density lipoprotein² and epidermal growth factor³ clustered selectively in these regions. The coated pit may also control some selection of its own lipid components as suggested by the apparent exclusion of cholesterol⁴.

Since all components of the plasma membrane of fibroblasts can be renewed the observations on the θ and H63 antigens means that such proteins must be taken up by other routes — otherwise the plasma membrane would act as a dead end for

redundant copies of the proteins. Perhaps the alternative route in this case is via uncoated membrane invaginations seen to sequester ferritin-labelled HLA antigens⁵. Coated pits observed in the same cells were free of marker.

The majority of proteins or peptides which enter the cell by endocytosis appear to be deposited in secondary lysosomes or multivesicular bodies (essentially the same thing). Do the associated receptors coincidentally suffer the same fate? At least one well-studied receptor⁶ — for epidermal growth factor — does appear to be destroyed together with its bound hormone⁶. For a number of other receptors, there is evidence of efficient recycling. Results obtained from studies of the hepatocyte galactose receptor and low density lipoprotein receptors in fibroblasts are exemplified by recent observations⁷ of the mannose/N-acetyl glucosamine receptor (macrophages).

Only a small fraction of the total cellular pool of sugar receptors are present on the macrophage surface at any time. However, if the accessible copies are destroyed enzymically at low temperature they can be rapidly and repeatedly replaced upon warming the cells, even in the absence of protein synthesis. Similar treatment of cells at 37°C quickly destroys the entire receptor stockpile suggesting that receptor turnover is fast and continues even in the absence of specific ligand. The galactose receptor in hepatocytes has a long intracellular half-life, of several days — an observation obtained after challenge with saturating amounts of specific ligand⁸.

For these receptors it is necessary to envisage recycling and to postulate that receptor and ligand part company somewhere between coated pit and secondary lysosome. Two recent articles describing the route of ligands entering cells via coated pits provide a new structural context for biochemical approaches to this problem.

In hepatic cells, Wall *et al.*⁹ have traced

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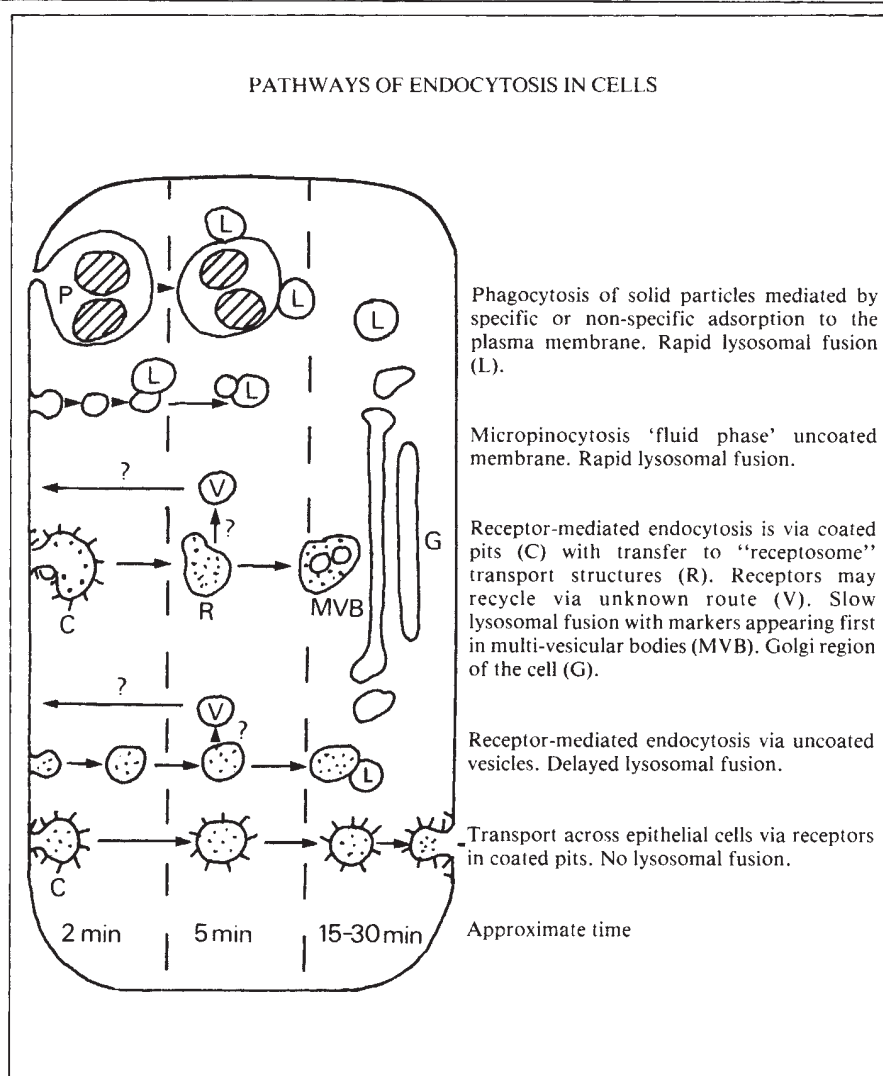
Michael Geisow is in the National Institute for Medical Research, Mill Hill, London.

the route of ligands expressing galactose residues. Numerous coated pits were seen in the absorptive membranes of cells before exposure to ligand. Coated vesicles close to the membrane were almost invariably shown to be pits lying with their principal axes roughly parallel to the plasma membrane as recently observed by others^{1,12}. Ligand first seen in these pits was found one and a half minutes later inside irregular structures (vesicles and tubules) lacking coats. After five minutes ligand appeared in the Golgi-lysosome region of the cell inside a heterogeneous population of uncoated structures. The appearance of ligand in association with lysosomal enzymes was first apparent at fifteen and complete at sixty minutes. Another recent report¹⁰ suggests the delay in fusion of pinocytic vesicles with lysosomes has a half-time of only seven minutes. Nevertheless, there does appear to be some agreement that ligand entering cells via coated pits takes longer to complete this standard journey than by other uptake routes¹¹.

Willingham and Pastan¹² present findings with strikingly similar features. In addition to electron microscopy, a system of image-intensified fluorescence microscopy permitted continuous observation of their ligand — α_2 -macroglobulin — as it was taken up by fibroblasts. They found no evidence of coated transport structures other than the initial coated pit. Instead the ligand transferred from pits to uncoated, irregular vesicles apparently like those described by Wall *et al.*⁹ with no clear transitional stage. The cytoplasmic structures were highly mobile and did not combine with anticlathrin antibodies; nor did they show any affinity for antibodies to the major microtubule or microfilament proteins. One more point of similarity between the Wall and the Willingham articles concerns timing. Label was in uncoated structures after five minutes, in the Golgi-lysosome region in fifteen minutes and in lysosomes after thirty minutes. To distinguish the uncoated vesicles arising as a result of endocytosis through coated pits from other pinocytic vesicles, Willingham and Pastan have coined the term 'receptosome'.

Concerning the new nomenclature, one might comment that the name would be even more appropriate if one could be sure that the receptors were still there. In the structures described by Willingham, the reaction product of their marker — conjugated peroxidase — did seem to be associated with the limiting membrane. However, the higher resolution marker employed by Wall *et al.* — conjugated ferritin — appeared throughout the lumen of the transporting vesicles at this stage. They speculate that dissociation of receptor and ligand might have already occurred.

Each of these recent articles emphasise the role of the clathrin-coated pit as a



structure specialising in receptor transfer across the plasma membrane. Strong evidence for a similar role has been reported for coated regions in endoplasmic reticulum membranes¹³. On the other hand direct evidence for coated vesicles as putative transport structures is as rare as coated pits are common. Recent reports either state or imply that the existence of coated vesicles, is extremely transient. Instead, the transport structures themselves appear to be smooth-membraned vesicles. It's worth noting that the isolation of coated vesicles containing ligand¹³ does not prove their independent existence as cytoplasmic entities, since homogenisation of plasma or Golgi membrane containing coated pits is very likely to produce closed vesicles when the membranes rupture.

The irregular appearance of the uncoated transport structures^{9,11} may imply intense membrane fusion activity — secondary lysosomes and Golgi condensing vacuoles are two examples of this. The 'receptosomes' of Willingham and Pastan might well act as a short limbo for incoming receptor-ligand bearing vesicles and, in the case of receptors which recycle, for

departure of vesicles bearing receptor alone. The delay in lysosomal sequestration of label entering cells by receptor-mediated endocytosis accords with the requirement for prior dissociation of ligand and receptor. However, entry via coated pits does not appear to be mandatory for a delay in lysosomal fusion, since HLA antigen complexes which are endocytosed in uncoated structures¹⁴ are also slow to appear in multi-vesicular bodies. The key to fusion or complete absence of fusion (as in the case of epithelial cells mediating transport across the cell layer) may lie in the proteins expressed upon the surface of the (uncoated?) transport structures.

I now anticipate the characterisation of 'Charonosomes' named after Charon who was able to return in his ferry after his passenger had crossed the point of no return into the underworld to face dissolution or immortality. But even more I await the direct, covalent labelling of a surface receptor. Surely, only a technique of this kind will enable the current models of receptor recycling and receptor-mediated endocytosis to achieve the elegance of the biological processes they seek to explain.

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The slow process of succession

from Peter D. Moore

THERE are certain areas of ecology which are made more difficult to investigate by the current tendency to limit research grants to three years. Take the long-term study of plant competition between bracken (*Pteridium aquilinum*) and heather (*Calluna vulgaris*) on the Brecklands of East Anglia. If Alex Watt (*J. Ecol.* **43**, 490; 1955) had been restricted to a three-year study period, plant ecology would be much the poorer. Then there is the study of successional processes, which may take several hundred years to reach completion. The subject is of considerable interest to both pure and applied ecologists but short-term studies have considerable weaknesses.

Some brief research projects concerning succession have been undertaken, such as that of Tramer (*Ecology* **56**, 905; 1975) who observed abandoned agricultural plots over a three-year period. But it is difficult to derive general statements concerning the nature of the process from such a restricted view. Many researchers have tried to solve this problem by observing the extant stages in successions which have been proceeding for different lengths of time. Classical studies such as those of Crocker and Major (*J. Ecol.* **43**, 427; 1955) on a glacial retreat recolonization and Olson (*Bot. Gaz.* **119**, 125; 1958) on the sand dunes of the Great Lakes region are of this type. Similar studies have used abandoned agricultural land of known history, such as those of Bazzaz (*Ecology* **56**, 485; 1975) and Nicholson and Monk (*Ecology* **55**, 1075; 1974). This approach suffers from the disadvantage that one must assume that study sites are alike in all but the developmental time factor. Naturally, this is hardly ever the case.

Ideally, one would like to take a single location and study the changes in plant and animal populations over many decades, or even centuries. In Britain we are fortunate in that one such experiment has been in progress for over a century, and some of the resulting data has now been analysed by Silvertown (*J. appl. Ecol.* **17**, 491; 1980).

This is the Park Grass Experiment at Rothamsted Experimental Station, which was begun in 1856 and which was originally intended to study the effects of various fertilizer regimes on grassland composition and productivity. The subdivision and replication of treatment plots was improved in 1964, but the data analysed by Silvertown covers only the period up to this revision.

From 1862, samples were cropped from the plots, were separated into species, dried and weighed. Plots which had received regular applications of ammonium sulphate were of lower pH and lower diversity (Shannon function), and all plots showed a negative relationship between overall biomass and diversity (and species richness).

The succession at Rothamsted has reached an equilibrium condition, under the constraints of harvesting imposed upon it. It is essentially a plagioclimax in the Tansley sense. Yet the variation of the equilibrium point in relation to nutrient capital and therefore biomass is of considerable interest for the theory of succession.

It adds considerable weight to the ideas of Green (*Biol. Conserv.* **4**, 378; 1972) who regarded the successional process in chalk grassland to be an accretion of nutrients in the biomass and litter, resulting in increased dominance and consequently reduced diversity among the plants. The results also fit well into the Grime model (*Nature* **242**, 344; 1973) if one regards increased fertilization as 'reduced stress', but the term over-simplifies the complexity of the system by reducing it to one dimension.

One of the particularly interesting features of the development of short turf into tall turf (increasing biomass) in grassland succession is that its influence upon invertebrate diversity is the reverse of that found in plants. As plant diversity falls, invertebrate diversity increases. This is considered by Morris (In *The Scientific Management of Plant and Animal Communities for Conservation*, ed. E. Duffey and A.S. Watt, Blackwell, Oxford, 527; 1971) to be a consequence of the increased opportunities for herbivorous grazers in the structurally and

microclimatically more complex environment offered by tall grassland plants. This, of course, will increase diversity at higher trophic levels also.

An Australian experiment has recently confirmed this relationship by monitoring the invertebrate faunas associated with different stock density of sheep. Hutchinson and King (*J. appl. Ecol.* **17**, 369; 1980) sampled sites over a three year period which were grazed at densities of 10 sheep ha⁻¹, 20 ha⁻¹ and 30 ha⁻¹. Apart from Scarabaeid beetle larvae and Oligochaeta, which showed greatest mean abundance (no. m⁻²) and biomass in intermediate grazing levels, other groups showed a reduction in biomass and density as the grazing pressure increased. Here again, grazing pressure can be considered as a controlling influence upon habitat structure.

But what of vertebrates? Small mammals, like invertebrates, are more abundant in taller, undisturbed turf (Duffey, *J. Anim. Ecol.* **31**, 571; 1962), but birds, particularly those which use grassland for feeding, seem to prefer short turf. In Sweden, Larsson (*Oikos* **20**, 136; 1969) has shown a considerable decrease in the numbers and diversity of birds frequenting the grassland areas around some Swedish lakes, following the removal of cattle grazing, and the consequent increase in vegetation cover.

This change in avifaunal diversity and density with the successional development from short to long turf has some interesting implications for applied ecology. A survey by Brough and Bridgman (*J. appl. Ecol.* **17**, 243; 1980) of thirteen airfields in Britain has shown very considerable differences in the density of feeding birds in short and long turf. The total counts over a two year period showed that three times as many birds overall used the short turf sites (which was defined as being a grass height of 5-10cm) when compared to long turf sites (grass height 15-20cm). In the case of gulls, the numbers feeding in long grass were only one fifth of those in short turf habitats. Their distaste for long turf may be associated with a greater difficulty in feeding upon soil invertebrates, coupled with a lack of visibility which might allow the approach of predators.

There thus seems to be a strong case for allowing the grassland succession on airfields to proceed beyond the short-turf stage and thus reduce the risk of bird strikes for aircraft. As Brough and Bridgman point out, however, there is one snag. The increased small mammal populations of the long grass may attract avian predators, such as kestrels and short-eared owls. But the data which they present suggests that these species are unlikely to reach seriously high population levels.

It would be pleasant to think that such a practical application of the study of what are essentially successional processes might encourage further and longer term studies.

Actin assembly

from Sarah E. Hitchcock-DeGregori

A survey of the literature shows that the study of actin filament assembly is positively booming although it is one of the older areas in the contractile protein field.

Understanding of the polymerization of many protein polymers grew out of biophysical studies begun in the 1960s on the mechanism of actin polymerization¹. The conversion of monomeric actin (G-actin) into filamentous actin (F-actin) has been viewed as a two-step condensation-polymerization process: condensation of a few actin monomers to form nuclei (the rate limiting step), followed by addition of monomers to form actin filaments (elongation). At steady-state, the F-actin is in equilibrium with a critical concentration of G-actin. Wegner² has proposed that actin elongates by a 'head-to-tail' mechanism, with a net addition of monomers to one end of the filament and a net depolymerization at the other end. While experimental work has supported the Wegner model, recent work suggests that the mechanism of actin assembly and disassembly may be more complex in detail³⁻⁵. The 'polymerizing' end of the filament has been shown to be the end that appears barbed in filaments decorated with myosin HMM or S₁^{6,7}. It is also the end that is attached to the Z-line in muscle, to dense bodies and to microvilli.

The model seemed particularly suitable to those working on muscle as in rabbit and frog skeletal muscle actin is in 1 μ long thin filaments, and once assembled with tropomyosin and troponin to this miraculously constant length, it seems to stay there until it needs to be turned over for new protein. Although many questions remained unanswered, other aspects of muscle contraction, such as regulation and myosin kinetics, received more attention in the 1970's.

This situation has now changed with the new emphasis on non-muscle motility. Bray and Thomas⁸ showed that a substantial amount of actin from cells is in a non-filamentous form. In addition, there have been numerous reports of changes in the state of actin which correlate with changes in cell shape and function.

It is now believed that the state of actin assembly is specifically regulated at a number of points, including (at a minimum) nucleation, addition of monomers at the net polymerizing end, loss of monomers at the net depolymerizing end (or other sites of depolymerization), and association of filaments to form bundles and networks. Nature has aided studies of these problems by supplying ready-made poisons, the cytochalasins and phalloidin, which interfere with one or more of these processes.

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First, the problem of nucleation. Although ionic conditions in the cell are highly favourable for polymerization of actin, much of it is non-filamentous. A protein called profilin that prevents the polymerization of actin has been isolated from a variety of sources^{9,14}. Profilin combines with G-actin and inhibits the formation of nuclei. The dissociation of profilin from actin is promoted by agents that are known to form sites of attachment for actin filaments in cells. For example, α -actinin is found in the Z-line and at other sites of filament attachment such as dense bodies in smooth muscle and adhesion plaques of stress fibers in cultured cells. Long suspected to be a promoter of actin polymerization, α -actinin has now been shown to dissociate profilin from actin, thereby allowing polymerization¹⁶. Similar results are found with spectrin-actin complex from erythrocyte membranes and with nuclei of purified actin^{12,17,18}. Once nucleation has taken place, elongation ensues, resulting in filament growth.

Assuming nucleating sites exist at all times in cells, an obligatory site of regulation is the elongation step. Cytochalasins and proteins with properties resembling cytochalasin are believed to regulate elongation. Research has focussed on the effect of cytochalasins on actin polymerization¹⁹. Cytochalasin binds to F-actin and spectrin-actin complex and inhibits polymerization, presumably by competing with the G-actin for the nucleating site. There is general agreement that, in terms of Wegner's head-to-tail model of elongation, cytochalasin inhibits polymerization by preventing addition of monomers to the preferred polymerization end, the barbed end of decorated actin filaments^{20,27}. This results in net depolymerization and shortening of existing actin filaments. In addition, some investigators believe cytochalasin may also alter or fragment filaments^{28,30}. It is probable that in the final analysis the mechanism of cytochalasin will be complex.

Recently, there have been reports of proteins with cytochalasin-like activities: from platelets³¹ and *Acanthamoeba*³². The platelet factor competes with cytochalasin B for spectrin-actin complexes, inhibits polymerization of G-actin on to these complexes or on to actin nuclei, and when added to preformed actin filaments causes a slow loss of viscosity. The *Acanthamoeba* factor appears both to promote nucleation and to inhibit the rate of elongation. There is electron microscopic evidence that in the presence of this protein, actin monomers are added at the pointed end but not at the barbed end of the filament. When the factor is added to filamentous actin, some depolymerization is observed. Isenberg *et al.* have interpreted these results to suggest

that their factor, which they call capping protein, may promote nucleation by stabilizing actin dimers and then inhibiting elongation by allowing growth only in the 'slow' or pointed direction. Capping protein (see this issue of *Nature*, p 455) and the platelet protein³³, like cytochalasin B, reduce the low shear viscosity believed to result from interactions between ends and sides of filaments and between filaments and other actin binding proteins^{27,28,34} and gelation factors^{35,37}. A factor with similar properties to capping protein has been isolated from conventional muscle actin preparations³⁸. These proteins may have a role in regulating the consistency of the cytoplasm.

From these reports it seems highly likely that capping or cytochalasin-like proteins may regulate the elongation of actin filaments on existing nucleating sites. This leaves us the problem of depolymerization of actin. Change in cell shape involves, in some cases, rapid dissolution of filamentous structures. From *in vitro* studies, it appears unlikely that factors blocking the net polymerizing end of actin (if this is the correct interpretation) or nucleation (profilin) could cause rapid depolymerization, since the ionic conditions of the cytoplasm are highly favourable for polymerization. Rather, it seems likely that there will be factors that actively depolymerize actin. DNase I depolymerizes actin rapidly, apparently by acting directly on the filament^{3,39}. Other factors that depolymerize actin in seconds or minutes *in vitro* have been isolated from plasma and serum^{40,42} and the presence of depolymerizing factors in cells has been reported^{42,43}. The detailed mode of action of these factors is the next problem that will have to be elucidated.

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Where do cosmic rays come from?

from a Correspondent

ABOUT once a second a single atomic nucleus carrying on its own at last 10 Joules of energy from Space hits an air nucleus in the Earth's atmosphere. It thereby initiates an enormous shower of secondary particles which builds up to an extensive air shower (EAS) of 10^{10} particles by the time it reaches sea-level. Such a primary cosmic ray particle has been accelerated somewhere in the Universe through the equivalent of a voltage difference of 6×10^{19} volts. How and where this acceleration takes place remain among the major unsolved questions of high energy astrophysics.

The origin of cosmic ray particles has been a subject of controversy for more than 50 years and has resulted in something of an East-West divide amongst astrophysicists. On the one side V. L. Ginzburg (P. N. Lebedev Institute of Physics, Academy of Science of the USSR, Moscow) and others have argued consistently that these cosmic ray nuclei originate mainly in our own Galaxy. On the other side, British theoretical astronomers, such as G. R. Burbidge (University of California), have maintained that the sources must be extragalactic. Whichever viewpoint is held, however, there is general agreement that supernovae explosions play a significant part in cosmic ray particle acceleration.

Most cosmic rays, of course, have energies far less than the highest energy particles; the spectrum at the Earth peaks at energies of 10^9 eV per nucleus and falls rapidly to energies greater than 10^{20} eV per nucleus (1 eV = 1.6×10^{-19} Joules). At the highest energies ($>10^{18}$ eV per nucleus) astrophysicists have so far failed to produce a satisfactory model for the acceleration mechanism. Almost by a process of elimination, however, the net seems to be closing in on possible source regions. Briefly the case is as follows. The ultra-high energy particles cannot originate and remain captured in the Galactic disc because the Galactic magnetic field is not strong or extensive enough to contain the particles. For a Galactic origin it is generally thought that strong anisotropy in the particle arrival directions should exist — presumably from the centre of the Galaxy. Again, the particles cannot come

from the deep depths of space. They could not travel that far without colliding with the Universal 2.7° K microwave background photons and undergoing strong absorption through pion production at energies greater than 10^{19} eV per nucleus. The experimental evidence shows that rather than a cut-off in the cosmic ray energy spectrum occurring at 10^{19} eV, it is just at this point that a flattening of the spectrum appears to occur. So where does that leave us?

The latest East-West confrontation took place recently in the beautiful old Russian capital of Leningrad where the VIIth European Cosmic Ray Symposium was held from September 15th-19th. Here A. W. Wolfendale (University of Durham) suggested that the problem could be resolved by a model in which those ultra-high energy particles reaching the Earth come predominantly from the nearby local supercluster of galaxies in Virgo. The actual sources would be active galaxies within the cluster of 2,500 galaxies. Wolfendale and his colleagues have developed their model to explain the available experimental data and it predicts an obvious anisotropy in the particles arrival direction at the Earth. Such an anisotropy in the general direction of Virgo is indeed observed for the highest energy particles detected by the Haverah Park EAS Detector Array in Yorkshire (*16th Int. Cosmic Ray Conf. Kyoto* **13**, 130; 1979).

At Leningrad J. Lloyd-Evans (University of Leeds) presented the latest data on the anisotropy analysis from the Haverah Park Group. These indeed confirm a sharp and significant change in the phase of preferred arrival direction from low Galactic latitudes to high positive latitudes as the primary energy increases above 10^{19} eV in EAS detected at Haverah Park. This change occurs sharply at 10^{19} eV per nucleus, just where the flattening in the energy spectrum is observed. So the Wolfendale model fits in well with the latest experimental data.

However, the 'extragalactic' Western scientists were not allowed to have it all their own way in Leningrad. D. D. Krasilnikov (Inst. of Cosmophysical Research and Astronomy, Yakutsk, USSR), presented a paper entitled

'Intensity Anisotropy of Highest Energy Cosmic Rays, its Energy Dependence'. In this the author considers the distribution on the celestial sphere of all the 313 cosmic ray particles with energies above 10^{19} eV detected by the four large EAS detector arrays in England, Australia, US and the USSR. Krasilnikov too demonstrates that the anisotropy increases and changes phase as the primary energy increases. In particular, he selects out for special treatment the particles with energy/ 3×10^{19} eV/nucleus. He splits the data in half in four different ways: (a) those coming from below and those above the Galactic equatorial belt of $\pm 30^\circ$ latitude; (b) those coming from inside and those outside the Galactic equatorial belt of $\pm 30^\circ$ from the Galactic centre; (c) those coming from opposite hemispheres pointing towards and away from the Galactic centre; and (d) those coming from opposite hemispheres pointing towards and away from the centre of the Virgo cluster. Taking the ratios of the intensities from the two groups in each of the four cases, Krasilnikov finds that only in case (c) does the intensity ratio ($= 0.43 \pm 0.14$) differ significantly from unity. Thus he claims the measured anisotropy is associated with Galactic co-ordinates. The author therefore concludes that these results point to a Galactic source for the particles and infer a mass composition of heavy or middle mass nuclei and a modulation of their propagation directions by Galactic disc and halo magnetic fields. He argues that although supernovae can only directly accelerate the particles up to $\sim 10^{17}$ eV per nucleus, further acceleration can be achieved by the magnetic fields of the Galaxy, both disc and halo, over time scales of 10^7 to 10^8 years.

Significantly, Krasilnikov needs heavy primary nuclei to support his theory. Unfortunately, as yet, there is little experimental guide as to the primary mass composition at these energies. What there is suggests that protons are still abundantly present as at lower energies. The East v West battle is thus still alive. We must now await the next round, to take place at the 17th IUPAP Conference in Paris next July.

The Leningrad symposium was, not unexpectedly, dominated by the Russians, since they combined the biannual European Symposium with an annual national cosmic ray meeting. The great magnitude of the Russian effort showed up forcefully in the special workshop session devoted to cosmogenic nuclides — that is the study of individual nuclear isotope concentration in meteorites. The analysis and interpretation of such data is now reaching a very high degree of sophistication.

ERRATUM

In the News and Views article "Lessons from a peptidergic neurone" by B. T. Pickering (13 November, **288**, 117; 1980) the molecular weights quoted were incorrectly altered by the insertion of 'K' and should instead be read in Daltons.

ARTICLES

First Voyager view of the rings of Saturn

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Pictures taken from Voyager 1 between 3 September and 13 October 1980 at a resolution of ~1,000 km/lp reveal new data on Saturn rings.

THE Voyager 1 spacecraft was launched on 5 September 1977 and successfully flew past Jupiter on 5 March 1979, assuming a trajectory to encounter Saturn on 12 November 1980. By mid-summer of 1980, at a range from Saturn of ~1 AU (1.5×10^8 km), the resolution of the Voyager Imaging System¹ had equalled that achieved in excellent seeing conditions with ground-based telescopes. Since then, pictures of the planet and its rings have revealed steadily increasing detail. We present here a preliminary discussion of imaging data for the rings at a resolution of about 1,000 kilometres per line pair (km/lp), corresponding to approximately 0.1 arcs as seen from Earth.

During the Voyager approach the spacecraft has viewed the planet from above (north of) the ecliptic plane, showing the rings open at an angle of ~9°. When seen from Earth at a similar opening angle, the surface brightness of the rings (illuminated by the Sun at about the same angle) is comparable to that of the planet. However, the rings as seen by Voyager are much darker, being illuminated at a low solar angle; the inclination to the Sun increased from zero on 3 March 1980 to 3.4° on 1 October.

The images we are using for the present analysis were obtained during the Voyager 1 Observatory Phase between 3 September and 13 October 1980. During that period the range to Saturn decreased from 92×10^6 to 40×10^6 km, and the resolution improved from 1,700 to 800 km/lp. The details of the ring are best shown in 3-s exposures with the clear filter (~300–650-nm bandpass). The photometric calibration of the Voyager cameras has been discussed in detail elsewhere². We follow the traditional nomenclature for the rings of Saturn given by Alexander³ and Cook *et al.*⁴, reserving the problem of naming recently observed structures within the rings until all of these features have been adequately defined.

Appearance of the rings

As the spacecraft has approached Saturn, finer radial structure in the rings has become apparent. At a range of ~1 AU (~3,000 km/lp), the Encke Division in the A ring was first clearly visible. At ~0.5 AU, the existence of a separate maximum within the Cassini Division was apparent, and two prominent minima in the C ring became visible. By the time the resolution was 1,000 km/lp, considerable fine structure between the major divisions was apparent, and the first evidence of azimuthal structure appeared in the central parts of the B ring. Figure 1 illustrates the ring as seen at this level of resolution.

Table 1 lists the radial features of the rings that can be seen at a resolution of 1,000 km/lp. The terms maximum and minimum refer to features, possibly unresolved, in the radial brightness distribution. At higher resolution it may be possible to deter-

mine if these relative minima are clear regions with widths below our 1,000-km resolution limit, or are simply zones of lower particle density. Similarly, the features we now describe as relative maxima may reveal internal structure (for example, zones of lower brightness) at higher resolution.

The most dramatic discovery from images at 1,000-km/lp resolution has been extensive azimuthal structure within the B ring, at distances of ~104,000–116,000 km from the centre of Saturn. These non-axisymmetric features take the form of lanes, which are seen near the ring ansae, approximately radial to the planet, and 5–10% darker than surrounding areas. The time resolution during Observatory Phase (one observation each 2h) is insufficient to follow the evolution of any particular marking, so we do not know the lifetimes of these features, except that some persist for at least 3 h. A more detailed discussion of the non-axisymmetric features will be given elsewhere.

Individual features

The centre of the Encke Division is at a distance of 133,400 km from the centre of Saturn ($2.21 R_s$, where $R_s = 60,330$ km⁵). It is barely resolved in the Voyager images, indicating a width of 1,000–2,000 km. The position agrees well with that measured in transmission by Gehrels *et al.*⁶ whereas the width seems somewhat greater than Gehrels' measurement (876 km).

The Cassini Division has a width of about 4,000 km and is centred at 118,900 km ($1.97 R_s$). It shows a nearly symmetrical brightness profile, as illustrated in Fig. 2. In the centre is a narrow ring, clearly separated from the A and B rings on either side. At current resolution this ring appears to be ~2,000-km wide. This ring also was seen by Pioneer, where it showed as a dark core in the bright Cassini Division; however, in diffuse transmission, as seen by Pioneer 11, it was ambiguous whether this feature corresponded to a local maximum or minimum in particle density. We now see that it is a maximum.

The most prominent features in the B ring, at this resolution, aspect and epoch, are three bright bands in the outer half of the ring, at distances of 102,300 km, 108,000 km and 116,300 km (see Fig. 2). Just outwards of the innermost bright feature is a minimum whose amplitude is time variable, which forms the inner boundary of the non-axisymmetric dark features that extend outwards almost as far as the outer edge of the B ring.

Inwards from the bright band (at 102,300 km) is a darker lane, followed by a fairly smooth decline in brightness to the edge of the B ring at 92,200 km. There is no indication of the deep minimum and prominent maximum near the inner edge reported by Dollfus⁷.

There are two prominent minima within the C ring, one at

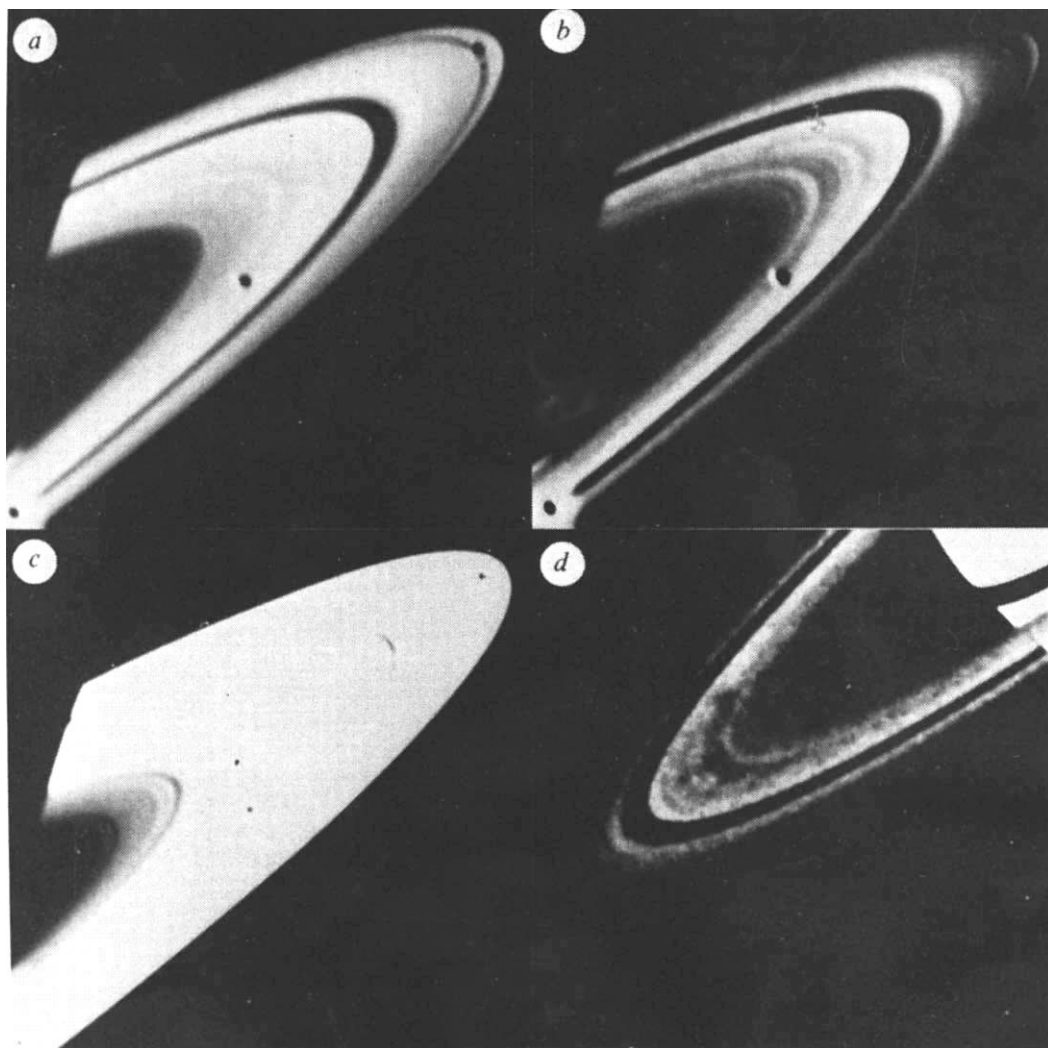
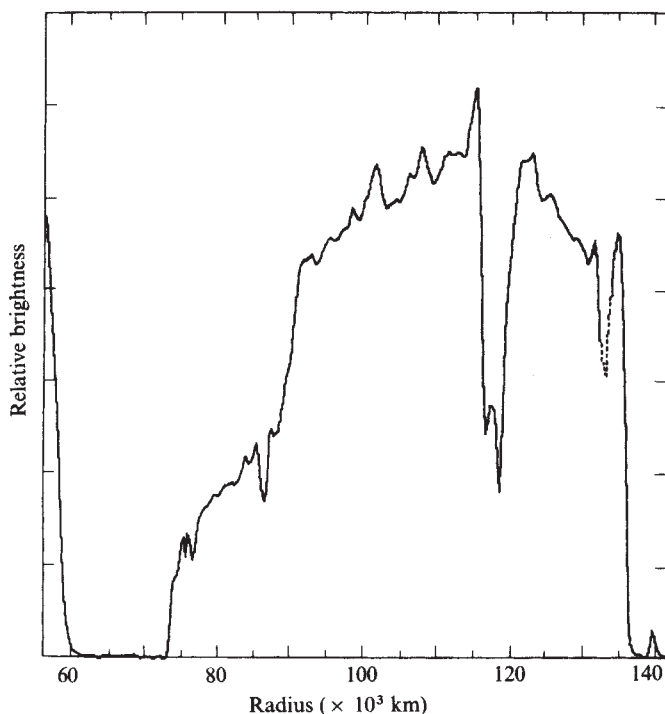


Fig. 1 *a*, *b* And *c* are three separate enhancements of a single Voyager 1 clear exposure (34032.33), taken on 13 October 1980 at a range of 40.5×10^6 km (resolution 750 km/lp). *a* Is enhanced to show the relative brightness of the major ring features: the C ring, B ring, Cassini Division (and material therein), A ring and Encke Division. *b* Has been enhanced to show radial structure within the A and B rings. In *c*, the darker C ring has been enhanced. *d* Is an image which was taken on 4 October 1980 from a distance of 52×10^6 km (resolution 960 km/lp) and illustrates the non-axisymmetric structure that has been discovered in the outer part of ring B.



86,700 km and one at 77,300 km. Because neither is fully resolved at 1,000-km resolution, we do not know how dark these gaps are. The inner minimum (77,300 km) has not been previously reported whereas the outer minimum corresponds closely to a feature observed by the Pioneer 11 IR investigation⁸ and apparently accounts for a feature seen in a Pioneer photograph of the ring shadow on the disk of Saturn. Gehrels *et al.*⁶ erroneously assumed that this minimum was at the boundary between the B and C rings and identified it with the brightness maximum in diffusely transmitted light at the outer edge of the C ring, at 90,000 km. We now see that there is no prominent minimum between these two rings, and the minimum observed by Voyager is $\sim 5,000$ km inwards of the B-C boundary.

The dimensions we obtain at a Voyager resolution of 1,000 km for the boundaries of the A, B, and C rings agree well with those obtained from ground-based observations^{4,9} and from Pioneer 11 (ref. 6).

Ring structure and satellite resonances

The radial structure in the rings has long been believed to result from the effects of gravitationally induced perturbations in ring

Fig. 2 The brightness profile of the rings at 750-km/lp resolution is shown here, using the same image as Fig. 1*a-c*. On the left is Saturn's terminator. The dotted line in the Encke Division (133,500 km) indicates the location of a resseau mark in the original data; here the profile is taken from an adjacent trace (that is, one just removed from the image's semi-major axis) through the data. The small peak at 140,000 km is the F ring.

particle orbits resulting from resonances with the inner satellites, particularly Mimas (see, for example, ref. 10). The satellites S10 and S11 (refs 11, 12), both orbiting near the edge of the ring at 1.51×10^5 km, provide potentially stronger perturbations than does Mimas. Other satellites may also have an influence on radial structure, particularly in view of the complex ring structure shown in the Voyager images. Because the difference in orbital longitude varies slowly for the two co-orbital satellites, S10 and S11 (ref. 13), ring structure may also have time-variable components.

Table 1 lists the assigned resonances for the various features in the rings. Table 2 lists all members of two types of resonance which fall within the rings. The satellites S10, S11, Mimas, Titan, Hyperion and Iapetus are the only ones which generate resonances of these two types within the rings. The resonances are characterized by multipliers of the mean orbital longitudes of the satellite, j' , and the particle in the ring, j . Resonances of Type I appear to the zeroth power in the orbital eccentricity of the satellite and involve $j' - j = 1$. Resonances of Type II, which are generally weaker, appear to the first power in the orbital eccentricity of the satellite and involve $j' - j = 2$. Note that resonances of Type I at $j' = 1, j = 0$ involve an equality of the rate of advance of the line of apsides of a ring particle and of the mean motion of the satellite. The motions of the apsides are those induced by Saturn's oblateness.

Table 1 Ring features

Feature	Position* ($\times 10^3$ km)	Position* (R_s)	Resolved	Possible resonance†
Saturn	60.33	1.00	—	—
Inner C	73.2	1.21	—	S10, S11 (II, I)
Maximum	74.5	1.23	No	—
Minimum	75.0	1.24	No	None
Maximum	75.5	1.25	No	—
Minimum	76.0	1.25	No	None
Maximum	76.3	1.26	No	—
Division	77.3	1.28	Yes?	Titan (I, 0)
Diffuse	83.0	1.38	Yes	Hyperion (I, 0)
Minimum				
Maximum	84.5	1.40	No	—
Minimum	85.3	1.42	No	None
Maximum	86.0	1.43	Yes	—
Division	86.7	1.44	Yes?	None
Maximum	88.3	1.46	No	—
Minimum	89.6	1.48	No	None
Maximum	90.1	1.49	No	—
Inner B	92.2	1.53	—	S1 (II, 2)?
Minimum	97.0	1.61	?	S10, S11 (I, 2)
Maximum	102.3	1.70	No	—
Minimum	104.6	1.73	Yes	None
Maximum	108.0	1.79	Yes	—
Minimum	111.0	1.84	Yes	None
Maximum	114.0	1.88	No	—
Minimum	115.0	1.90	No	None
Maximum	116.3	1.93	No	—
Inner	117.5	1.95	—	Mimas (II, 2)
Cassini				
Center	118.9	1.97	—	—
Cassini				
Outer	121.0	2.01	—	S10, S11 (II, 5) Iapetus (I, 0)
Cassini				
Maximum	124.5	2.06	Yes	—
Minimum	126.6	2.10	No	S10, S11 (II, 6; I, 3; II, 7)
Minimum	131.0	2.17	No	S10, S11 (II, 8; I, 4)
Encke	133.5	2.21	Yes	S10, S11 (II, 9; II, 10; I, 5); Mimas (II, 4)
Division				
Outer A	136.2	2.26	—	S10, S11 (II, 12; I, 6)

Region of azimuthal structure ranges from 1.72 to $\sim 1.92 R_s$.

* All ± 500 km, except those underlined, which are $\pm 1,000$ km.

† Notation for resonances consists of Type (I or II) and multiplier j of longitude of ring particle in argument of libration (see Table 2).

Table 2 Resonances with known satellites

Satellites	Type	j'	j	Distance (R_s)	Comments
S10, S11	II	3	1	1.227	Inner edge, C
Titan	I	1	0	1.289	Gap (1.28)
Hyperion	I	1	0	1.396	Seen? (1.38)
Mimas	II	3	1	1.495	Not seen
S10, S11	II	4	2	1.591	Not separable from next
	I	2	1	1.595	See (1.61)
	II	5	3	1.792	Not seen
	II	6	4	1.921	Not separable from next
Mimas	I	3	2	1.922	Not seen
	II	4	2	1.945	Not separable from next
	I	2	1	1.948	Inner part of Cassini Division (inner edge)
S10, S11	II	7	5	2.010	Not separable from next
Iapetus	I	1	0	2.010	Outer part of Cassini Division (outer edge)
S10, S11	II	8	6	2.075	Not separable from next
	I	4	3	2.076	
	II	9	7	2.125	Seen (2.10)
	II	10	8	2.166	Not separable from next
Mimas	I	5	4	2.166	Seen (2.17)
	II	5	3	2.193	In Encke Division
S10, S11	II	11	9	2.199	In Encke Division
	II	12	10	2.225	Not separable from next
	I	6	5	2.225	In Encke Division
	II	13	11	2.247	Not seen
S10, S11	II	14	12	2.267	Not separable from next
	I	7	6	2.267	Outer edge, A

Tables 1 and 2 show that as the density of matter in ring C increases outward, the divisions due to S10 and S11 disappear; this happens first for resonances of Type II, then for those of Type I. Those of Type I reappear outside the Cassini Division and may form the outer edge of ring A. For Mimas there is good evidence only for resonances of Type I. The Type I apsidal resonance from Iapetus may contribute to structure within the Cassini Division. Hyperion's small size casts doubt on the assignment listed.

It is clear from Tables 1 and 2 that the classical satellite resonances do not explain most of the fine structure seen in the rings. Some of the structure may be generated by the presence of small, as yet undiscovered satellites within the rings, for example, at the edges of major divisions. These satellites may produce additional maxima and minima through their orbital resonances; they may also serve as sources for some of the ring material. The presence of the spoke-like azimuthal structures in the B ring reminds us that other dynamical processes may also be important in producing observed features.

Preliminary ring photometry

The ring brightness is of great interest, as it contains implications as to the constituent particle albedos and phase functions. Several previous studies^{14,15} have been conducted to determine these parameters. We have made preliminary model calculations of ring brightness using a doubling-adding radiative transfer code¹⁶ for the geometry of these observations. Although the data reduction is as yet preliminary, certain overall conclusions may be drawn.

Due to the low Sun angle ($\sim 3^\circ$), the dependence of observed brightness on layer optical depth is small. We find that the constituent particles of the A and B rings are characterized by a greater degree of diffuse backscattering ability than previously

suspected. In fact, particles behaving like Lambert spheres provide quite a good fit to the observed A- and B-ring brightness.

However, the C ring has noticeably different properties. For an optical depth ~ 0.1 (refs 15, 17), the C ring would be nearly as bright as the A and B rings at the present epoch, if the constituent particles were identical. However, Voyager observations show it to be less than half of the A and B brightness. Fuller phase-angle coverage, obtained subsequently in the mission, will be required for a distinction to be made between albedo and phase-function effects. However, the unambiguous implication is that the C-ring particles are different, either darker or more highly forward scattering than the A- and B-ring particles. This qualitative conclusion is consistent with the conclusions of recent analyses of Pioneer Saturn data^{15,18}. This observation argues very strongly for a different particle population in the C

ring as compared with the main rings. More data and analysis will refine these preliminary conclusions.

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Note added in proof: Subsequent Voyager observations have shown the azimuthal features which appear dark in back-scattered light reverse contrast and appear bright against the background B ring in forward-scattered illumination. This behaviour indicates that these features result from concentrations of small particles (a few micrometres) and not from variations in the B-ring particle density.

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Planform of mantle convection beneath the Pacific Ocean

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The correlation between variations of the bathymetry and of the geoid in the Pacific Ocean suggests that both are the surface expression of mantle convection. The planform of the convection which is required to generate the observed anomalies does not consist of rolls but is three dimensional, with rising and sinking jets elongated in the direction of motion of the Pacific plate relative to the frame of reference defined by the hot spots. The spacing between the maxima and minima of the geoid is between 1,500 and 2,000 km and hence favours a model of mantle convection with multiple scales of circulation.

ALTHOUGH most geophysicists agree that plate motions are maintained by mantle convection, there is little agreement about the geometry of the motion which converts heat into work. One of the principal difficulties is that the motion of the plates themselves provides a few restrictions on the form of the flow, because their motion can be described by rigid body rotations. Runcorn¹ recognized that long-wavelength gravity anomalies should be directly related to the fluid motion, and McKenzie *et al.*² showed that upper surface of the convecting region with constant viscosity was elevated, producing a positive gravity anomaly, over the rising region of the flow. The influence of the elastic forces within the plate on the magnitude and sign of the anomalies of gravity and bathymetry above a convecting region^{3,4} is small if their wavelength is > 500 km and the elastic thickness of the plates is ≤ 30 km (ref. 5). The wavelengths are expected to be at least twice the depth of the convecting region, or $> 1,400$ km, if the convection is confined to the upper mantle. The amplitudes of the gravity and bathymetry anomalies obtained from the numerical calculations are about 20 mGal and 500 m respectively. Hence the anomalies should be easily observed, correlated with each other, and be little affected by the elastic behaviour of the plates. Two difficulties must be overcome before geophysical observations can be used to examine the convective planform. The first involves corrections to the observed bathymetry to remove the effects of lithospheric

age, determined from the magnetic anomalies, and of sediment loading. Both of these can now be calculated with reasonable accuracy, and the depth obtained after these corrections has been applied is known as the residual depth. Only that part of the residual depth which is not produced by variations in crustal thickness and whose wavelength is longer than ~ 500 km is dynamically maintained by the convection. The other difficulty concerns the gravity field. Surface observations contain large amplitude short wavelength anomalies, and long wavelength errors are produced by drift of the instrument and calibration errors. For these reasons the long wavelength gravity anomalies can only be reliably obtained from surface ship observations in areas where there is dense coverage.

Historical techniques

The first attempt to use these arguments to investigate mantle convection⁶ used the depth of the ridge axis and a satellite-derived gravity field. The procedure avoided the use of depth corrections, as the age of the ridge axis is the same everywhere and the sediment cover is very thin. Although the correlation between gravity and residual depth was clear in the North Atlantic, where it agreed with the numerical calculations, it was much less convincing in the other oceans. This behaviour was probably produced by errors in the satellite gravity field, whose

higher harmonics were poorly determined. Extensive two-dimensional investigations of gravity and residual depth anomalies in the North Atlantic⁴ and the Pacific around Hawaii⁷ using surface gravity observations showed good agreement with the numerical models but neither covered a large enough area to demonstrate the convective planform. Cochran and Talwani⁸ considered a larger region but failed to find a global relationship between gravity and residual depth similar to that found in the smaller regions^{4,7}. Their lack of success was probably due to the poor sampling of the long-wavelength part of the gravity field due to the wide spacing of the ship tracks. Figure 3 shows that the amplitude of the gravity anomalies corresponding to 10 m variations in geoid height is only ~ 20 mGal. Such gravity

anomalies will be difficult to detect in the presence of short wavelength anomalies of hundreds of mGal produced by seamounts, and measurement errors of at least 10 mGal. Menard and Dorman⁹ attempted to use the bathymetry alone to examine the convective planform. Because long-wavelength variations in bathymetry may be isostatically compensated, and hence be unrelated to mantle convection, it is not obvious what constraints a study of the bathymetry alone imposes on the form of the circulation (see Fig. 2). An attempt¹⁰ to obtain a gravity field over the Pacific from satellite orbits and surface gravity seems to have produced spurious anomalies, due to lack of sufficient surface observations and an instability caused by downward continuation¹¹.

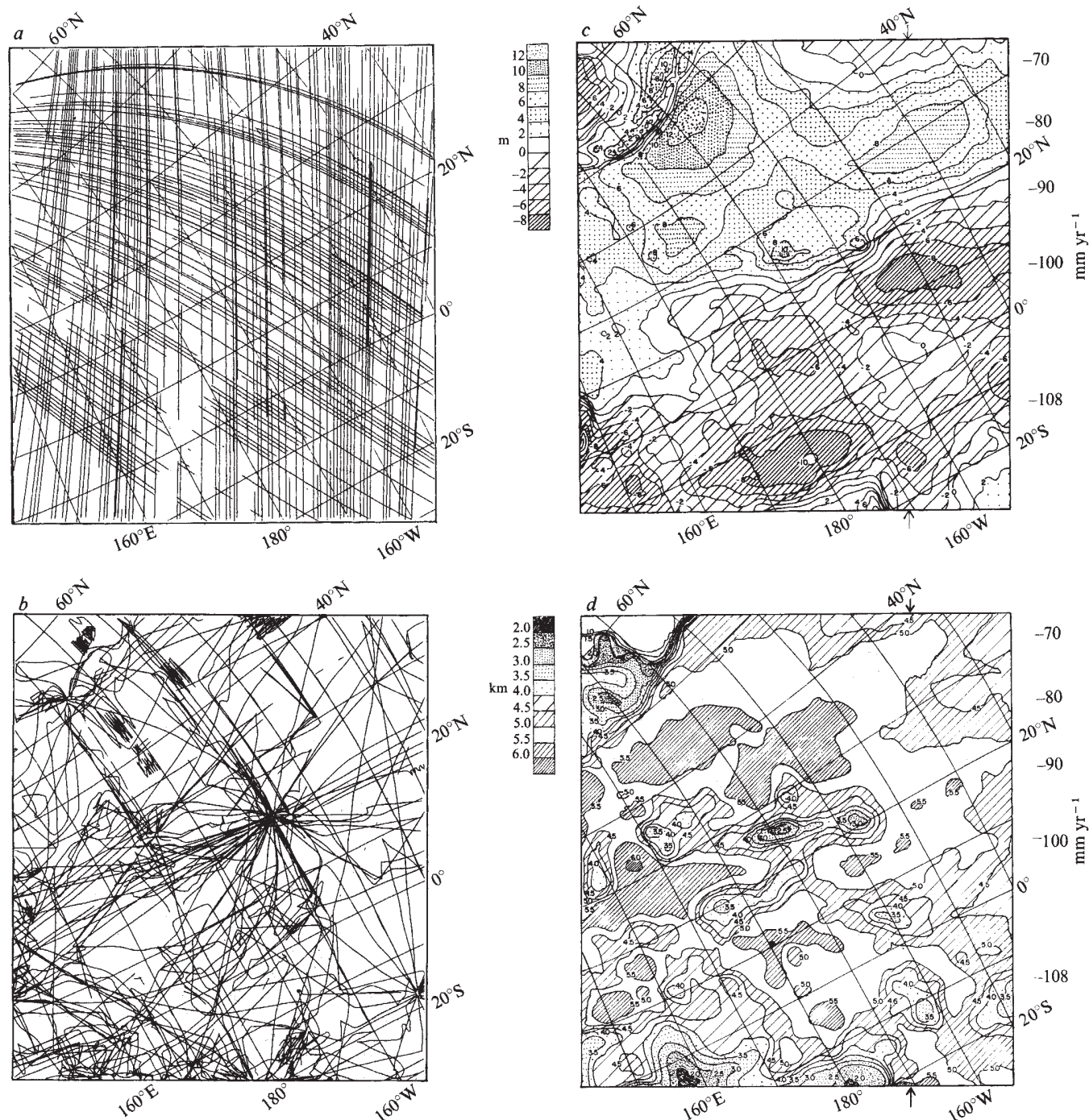


Fig. 1 Satellite altimeter (a) and ship (b) tracks used to obtain contour maps of geoid (c) and bathymetry (d) in the North and Central Pacific. The projection is an oblique mercator projection with axis 61.7°N , -82.8°E . Contour intervals are 2 m for (c) and 0.5 km for (d). The grid spacing in both cases is 100 km at the equator of the projection, and the gaussian half width for interpolation is 110 km for (c), 150 km for (d). The velocities shown on the right of (c) are those of the Pacific plate, moving from right to left, relative to the hot spot frame¹⁵, and is 108 mm yr^{-1} at the equator of the projection.

A detailed analysis of the gravity field and surface deformation above different types of convection¹² showed that their ratio depends little on the form of the heating, the Rayleigh number, or the boundary conditions. Hence little further progress could be made until more accurate estimates of gravity anomalies between wavelengths of 500 km and 5,000 km became available over large areas of the oceans. GEOS 3 has now provided such estimates by direct determination of the geoid.

GEOS 3 measurements

The GEOS 3 satellite contains a radar altimeter which measures the distance from the satellite to the sea surface. If the orbit of the satellite and oceanographic corrections are known the geoid can be obtained directly. In practice, the precision of the altimeter is between 0.5 and 1 m, and analysis of the cross-over errors between different tracks shows errors of ~ 10 m (ref. 13) due to orbit errors. The orbit errors can be reduced to about 0.6 m by adjusting the orbits to minimize the cross-over errors¹³. We used these adjusted heights. Oceanographic corrections consist of tides and both time-dependent and steady-state variations produced by currents. These corrections are poorly known, but are probably < 1 m in most parts of the ocean and were therefore ignored. The heights h_i corrected for bias and trend¹³, at points described by radial unit vectors $\mathbf{a}_i$ were then filtered with a gaussian filter to give a smoothed height H_i at position $\mathbf{b}_i$

$$H_i = \sum_j h_j \omega_j / \sum_j \omega_j, \quad \mathbf{b}_i = \sum_j \mathbf{a}_j \omega_j / \sum_j \omega_j$$

$$\omega_j = \exp(-d_j^2/\sigma^2) \quad (1)$$

where d_j is the distance between a chosen point on the profile and $\mathbf{a}_j$, and σ is the half width of the gaussian filter, chosen to be 100 km. In addition a weight W_i was obtained from the ratio of the rectangular approximation to the integral of the filter to the true integral

$$W_i = S \sum_j \omega_j / \sigma \sqrt{\pi}$$

where S is the spacing between successive points on the profile. If $W_i < 0.3$ the point was rejected. This processing reduced the number of data points while leaving unchanged longer wavelength features of the geoid. Because the geoid is dominated by long wavelength anomalies ($\lambda > 5,000$ km) and we are principally interested in those of intermediate wavelength, we subtracted the GEM7 geoid¹⁴ up to and including $l = m = 10$. GEM7 was used because this geoid was determined from satellite orbits alone, and because the coefficients of degree and order < 10 agree excellently with those of more recent determinations. The points $\mathbf{b}_i$ were projected onto a plane to give a position $\mathbf{x}_i$, and H_i then interpolated onto a square mesh using equation (1) with $\sigma = 110$ km, d_j being the distance between a mesh point and $\mathbf{x}_j$ in the plane of the projection, provided $d_j < 4\sigma$. Otherwise ω_j was set to zero. Finally the grid values were machine contoured. The bathymetry obtained from digitized profiles of more than 400 cruises in the Pacific Ocean treated similarly, except for the interpolation onto a square mesh where $\sigma = 150$ km was used.

Results

Figure 1 shows contour maps of the geoid and bathymetry, together with the satellite and ship tracks used. The area was chosen because it is large and well studied. Except in the upper right-hand corner of Fig. 1 sediment thicknesses are thin and uniform, and hence sediment loading corrections, which were not made, will produce little change in Fig. 1d. The depths in Fig. 1 have also not been corrected for subsidence as the lithosphere cools. Such corrections are also only important in the upper right-hand corner of Fig. 1, because the remainder of the area studied is underlain by lithosphere older than 60 Myr. The projection is an oblique mercator projection with the pole of relative motion between the Pacific plate and the hot spot frame

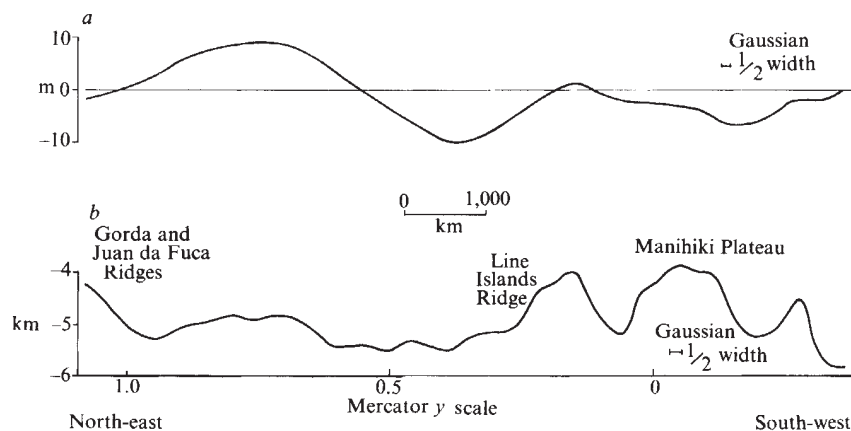
as an axis¹⁵, and the mesh spacing in Fig. 1c, d is 100 km on the equator of the projection. The region shown in Fig. 1 covers most of the North and Central Pacific. An accurate short wavelength anomaly produced by the Aleutian Island Arc is clearly visible at the top left of Fig. 1c, the anomaly on the left-hand edge is produced by the Mariana Island Arc, and those along the bottom by the Solomon and Tonga arcs. The geoid contours show the existence of elliptical maxima and minima whose amplitude is about 8 m and whose separation is between 1,500 and 2,000 km. One of the largest of these anomalies is associated with the Hawaiian Swell and has already been mapped using ship profiles⁵. The anomalies in Fig. 1c are too large and their separation is too great to be supported by the elastic part of the lithosphere, and therefore they must be dynamically maintained by mantle convection. Figure 1d shows that many of the geoid anomalies in Fig. 1c are associated with bathymetric anomalies. Figure 2 shows two profiles obtained from the values of the geoid and the bathymetry on the mesh points and plotted against distance in the oblique mercator projection to show the correlation more clearly. These two profiles are in the eastern part of the Pacific, where the geoid anomalies are large. The shoal region at the northern end of the profile is produced by the young lithosphere near the Gorda and Juan da Fuca ridge crests. This bathymetric anomaly does not correlate with the geoid and will be removed by the age corrections which have not yet been applied. Such corrections will not remove depth variations which are maintained by variations in crustal thickness, produced by vulcanism either on ridge axes or within plate interiors. Two such features, the Line Islands ridge and the Manihiki Plateau, are crossed by the profiles in Fig. 2 and both were produced by ridge axis vulcanism. Neither the crustal thickness nor the density of such ridges is well known, and therefore corrections are hard to apply. Towards the trench systems in the west the geoid is flatter than in the east, and several small anomalies of 2 and 4 m are associated with some of the large aseismic plateaus, such as the Shatsky and Hess Rises. Ridge axis vulcanism, which builds aseismic ridges at or above sea level on thin lithosphere, gives rise to anomalies of this magnitude, and there are many such features in the western Pacific¹⁶.

The geoid anomalies in Fig. 1c are mostly elliptical, with the long axes of the ellipses approximately parallel to the x axis of the plot. As the projection is an oblique mercator projection whose axis is the pole of relative motion between the Pacific plate and hot spot frame, the rigid motion of the Pacific plate in Fig. 1c corresponds to a translation in the x direction. The relative velocity in this projection is $v_0 \operatorname{sech} y$, where v_0 is the equatorial velocity and y is the vertical coordinate of the mercator projection, and is shown on the right of Fig. 1c. The ellipticity suggests that the convective pattern is elongated in the direction of motion. The contours provide evidence that the elongation increases with increasing velocity.

Discussion

The above observations provide various constraints on models of mantle convection. Perhaps the most important is that the geoid strongly suggests the existence of mantle convection whose horizontal scale is considerably smaller than that of the plates themselves, and that the elongation of the anomalies in one direction results from the shearing of the circulation by plate motions. Such a model has previously been proposed on theoretical grounds^{3,17}; where it was argued that the spacing between the rising and sinking regions should be similar to the depth of the convecting layer, and that the planform of the flow would probably consist of rolls aligned with their axes in the direction of motion, at least beneath rapidly moving plates such as the Pacific. This conclusion was qualified by Skilbeck and McKenzie¹⁸ who used an approximate method to examine the stability of a model suggested by Richter and McKenzie¹⁹, which possessed a low viscosity zone beneath the plates which decoupled the horizontal movement of the plates from that of the mantle below. The calculations suggested that even the Pacific

Fig. 2 Profiles of the geoid (*a*) and bathymetry (*b*) between the arrows on Fig. 1c, *d*. The horizontal axis shows the *y* coordinate in the oblique mercator projection, and the scale applies at *y* = 0.



was unlikely to be moving sufficiently fast to stabilize rolls, and that the small scale flow would probably be three dimensional. An alternative explanation is suggested by the experiments of Richter and Parsons¹⁷ on the evolution of sheared convection. As the geometry of the Hawaiian Ridge requires the motion of the Pacific plate in the hot spot frame to have changed ~40 Myr ago, the present direction of motion may not have existed for long enough to have established rolls. It is obviously of interest to extend the observations of Richter and Parsons¹⁷ to slower shearing rates.

Figure 3*a*, *b* and *c* shows the geoid, bathymetry and gravity produced by the convective flow in Fig. 3*d*, *e*. The depth of the convective layer is 700 km, the Rayleigh number is 1.4×10^6 , and the flux is fixed at 58.5 mW m^{-2} on both boundaries (see refs 2 and 20 for details). Both horizontal and vertical scales are the same as those in Fig. 2 at the equator of the projection. Both the horizontal scale and the amplitude of the bathymetry and geoid in Fig. 3 are similar to those in the Pacific. Superimposed on the cellular circulation is a time-dependent flow driven by hot and cold blobs detaching from the boundaries. This flow produces obvious bathymetric and gravity anomalies, but the wavelength is too small to produce more than 1 m anomalies in the geoid. Similar numerical experiments with the more realistic boundary condition of a plate superimposed on top of the convecting region also show that the spacing is often considerably greater than the layer depth (G. Houseman, personal communication). Hence the geometry suggested by Fig. 1 is to be expected, and is not evidence for whole mantle convection.

An unexpected feature of the geometry of the geoid anomalies is that they are larger in the east where the plate is young than they are in the west where it is old. This distribution suggests that they are not the result of instabilities beneath the cooling plate, and hence are not related to the flow postulated by Parsons and McKenzie²¹ to account for the observed relation between depth and age. The anomalies are better explained by instabilities of a lower hot boundary layer, perhaps at the base of the upper mantle. Near the trenches, where cold material is returned to the base of the convecting layer, the lower boundary layer is stable. As this material moves towards the east it is warmed by heat conducting into the upper mantle from the lower mantle, and becomes unstable. The same behaviour is clearly seen in the right-hand cell in Fig. 3*d*. The geoid anomalies are the surface expression of these instabilities.

Transfer of heat to the base of old lithosphere could still be produced by small cold blobs detaching from its base, similar to those on the right of Fig. 3*d*, because the geoid anomalies they would produce would be similar in magnitude to the noise (Fig. 3*a*). Such blobs would, however, produce obvious anomalies in the gravity field (Fig. 3*c*).

Some of the existing models of the return flow on a spherical Earth²²⁻²⁴ show that the return flow is approximately antiparallel to the plate motion, and therefore can account for the elongation of the geoid anomalies in Fig. 1. All these models, however, neglect the thermal buoyancy forces in the interior of

the convecting region. As such forces are dominant within large aspect ratio convective cells, such as that on the right in Fig. 3, the direction of flow obtained from the model calculations may not agree well with that in the mantle.

Conclusions

The observations discussed above have little relevance to whether the convective circulation involved in plate movements is confined to the upper mantle or extends throughout the whole mantle. If the whole mantle is convecting its Rayleigh number must be 10^9 – 10^{10} and the flow must be principally driven by internal heating. In these conditions the horizontal scale is

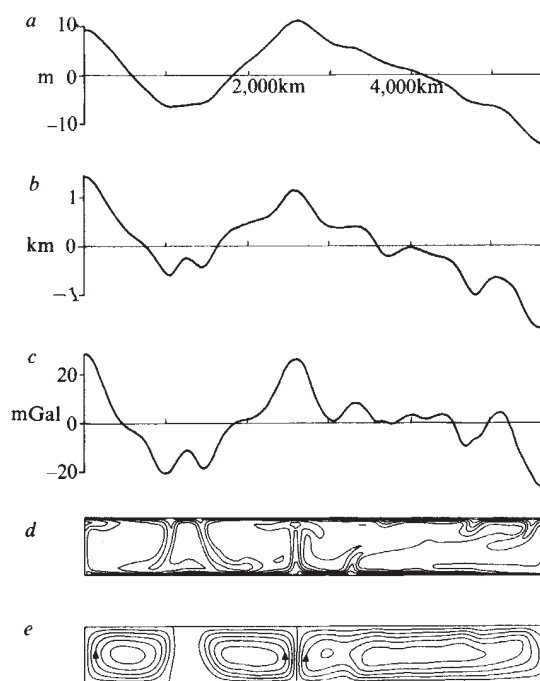


Fig. 3 Profiles of the geoid (*a*), bathymetry (*b*) and gravity (*c*) above a convecting fluid where the planform of the convection consists of two-dimensional rolls normal to the plane of the page. In all three cases the surface deformation obtained by the method outlined in ref. 12 has been convolved with the response of an elastic plate 30 km thick³. The horizontal scales are the same as those in Fig. 2 at the equator of the projection, and the vertical scales are the same. The temperature (*d*) contoured at 100°C intervals and the stream function (*e*) at 0.1 intervals are for a Rayleigh number of 1.4×10^6 with both upper and lower boundaries stress free and deformable. All the heat is supplied from below and the heat flux, not the temperature, is fixed on both boundaries (see ref. 20 for details).

controlled by the thickness of the boundary layer, and may be much smaller than the depth of the layer itself. In contrast, if the circulation is restricted to the upper mantle the boundary conditions²⁰ may produce a circulation whose horizontal scale is considerably greater than its depth (Fig. 3). Hence the horizontal scale is very little guide to the vertical extent of the flow. Nonetheless, the geometry of the anomalies is more easily explained by instabilities of hot lower boundary layer, which exists only if heat is supplied from below. Although the existence of such a boundary layer is required by models in which the flow is confined to the upper mantle, it is possible, though unlikely,

that the unstable layer is as deep as the core-mantle boundary and that a considerable fraction of the heat is supplied from within the core. There is some evidence that models in which all the heat is supplied by internal heating are not compatible with the anomaly distribution, because instabilities can only originate in the upper boundary layer. The presence of large anomalies over the younger but not the older part of the Pacific suggests that the instabilities arise in the lower, not the upper, boundary layer.

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Vesiculation of mafic magma during replenishment of silicic magma reservoirs

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Mafic inclusions are lower in bulk density than the andesitic and dacitic lavas in which they occur, and apparently represent a foam inflated during rapid cooling of wet mafic magma in contact with cooler, more silicic reservoir magma. This process occurs as mafic magma enters the base of the reservoir, so that the mafic/silicic interface becomes unstable in Rayleigh-Taylor fashion. Mixing in these reservoirs thus depends on water content of mafic magma and pressure.

MAFIC inclusions in andesitic and dacitic lavas and in equivalent plutonic rocks have been interpreted as cumulates from fractional crystallization¹, restites from deep crustal melting² and rapidly crystallized mafic magmatic material generated during magma mixing³. The implications of these interpretations for both magmatic and crustal evolution differ dramatically. I have previously argued that the inclusions result from magma mixing on the basis of their texture, bulk composition and phase composition⁴⁻⁶. A new observation is that the inclusions exhibit high vesicularity and consequently have a lower bulk density than their hosts. This property is consistent with generation of the inclusions by magma mixing, and may play an important part in the evolution of large magma reservoirs within the upper crust.

Origin of mafic inclusions

Intermediate composition calc-alkaline volcanic and plutonic rocks commonly contain fine-grained mafic inclusions which appear as dark clots or blobs ranging from a few millimetres to a few tens of centimetres in diameter. Despite differing geological settings of the igneous complexes in which they occur, these inclusions display remarkably uniform textures, compositions and physical properties. In volcanic rocks the inclusions are dominantly plagioclase + hornblende + glass or plagioclase + pyroxene + glass. In plutonic rocks, a felsic matrix is present instead of glass and the dominant mafic phase is usually hornblende or biotite. Several observations indicate that the

inclusions represent mafic magma injected into and chilled within cooler, more silicic magma reservoirs.

(1) Most inclusions are subspherical in shape, unlike accidental xenoliths derived from solid or nearly solid wall rock.

(2) In bulk composition and phenocryst content (typically calcic plagioclase and magnesian olivine) inclusions resemble associated mafic lava. This should not be the case for cumulates, the composition of which is controlled by the crystallizing magma and physical properties of the precipitating phases.

(3) Inclusions have an igneous texture characterized by abundant, elongated, strongly zoned, euhedral crystals in residual glass, indicative of rapid cooling and crystallization to just above solidus. This texture differs from the glassy or microcrystalline groundmass characteristic of mafic magma quenched at the surface. It also differs from the partial melt texture to be expected in restites.

(4) Some large inclusions exhibit outwards decrease in grain size, increase in elongation of grains, and/or an outer hybrid rind containing reacted or resorbed phenocrysts from the host lava. These observations indicate that the inclusions were chilled against the host, but that both host and inclusions were molten at the same time.

(5) Phenocrysts in the host lava are reacted or resorbed, consistent with heating of reservoir magma during cooling and crystallization of the inclusions.

These observations are further discussed elsewhere⁴⁻⁶. Important implications are that crustal magma reservoirs are

Table 1 Densities of some mafic inclusions and their host lavas*

Volcano	Sample description	Inclusions (g cm^{-3})		Nonvesicular host lava (g cm^{-3})
		Bulk (ρ_X)	Grain (ρ_B)	
Medicine Lake Highland, California	Rhyolite to pyroxene dacite, Glass Mountain flow	$2.15 \pm 0.04^\dagger$	2.79 ± 0.01	$2.38 \pm 0.01^\ddagger$
Crater Lake, Oregon	Hornblende dacite, Llao Rock flow	1.87 ± 0.03	$2.79^\S$	2.38 ± 0.01
Lassen Volcanic National Park, California	Hornblende dacite, Chaos Crag domes	2.16	2.75	$2.47^\parallel$
Jemez Mountains, New Mexico	Hornblende dacite, Chicoma Mtn	2.07 ± 0.01	2.76	2.46 ± 0.04

* To obtain bulk density, 0.5–1.0-kg samples of inclusions were separated from host lavas and weighed in air and water. To obtain grain density, these samples were dried and crushed to destroy at least 99% of voids, as indicated by inspection of polished grain mounts. The powder was then weighed in air and water, with the wet measurement made following subjection of the powder to a vacuum for 20 min and addition of water while under vacuum.

† Standard deviation given where $N \geq 3$.

‡ Rhyolitic portion of flow.

§ Estimated from ρ_X and point count of voids. Voids were too small to remove by crushing.

$^\parallel$ Grain density of vesicular surface phase.

maintained by convective transport of heat from the mantle and contain a mixture of components of crustal and mantle derivation⁵.

Vesicularity of inclusions

Inclusions in lavas contain abundant, fine, evenly distributed vesicles (Figs 1 and 2), even where the host lava contains virtually no vesicles (Fig. 2). Where vesicularity varies within a volcanic unit, as in lava flows with a dense interior and pumiceous surface, vesicularity of inclusions seems to be nearly independent of vesicularity of the host⁷.

Although vesicles are common in extrusive materials, the uniformity in vesicular texture of the inclusions is remarkable. Vesicles in tephra and lavas vary greatly in size and shape (spherical, elongate or irregular) and are sometimes unevenly distributed over distances of 1 cm or less. Vesicles are generally not present in accidental xenoliths. In contrast, vesicular textures in mafic inclusions are consistent not only within a single flow, but among flows of completely unrelated volcanoes (Fig. 1).

Measurement of bulk and grain density of the inclusions (Table 1) shows that this textural similarity is also reflected in

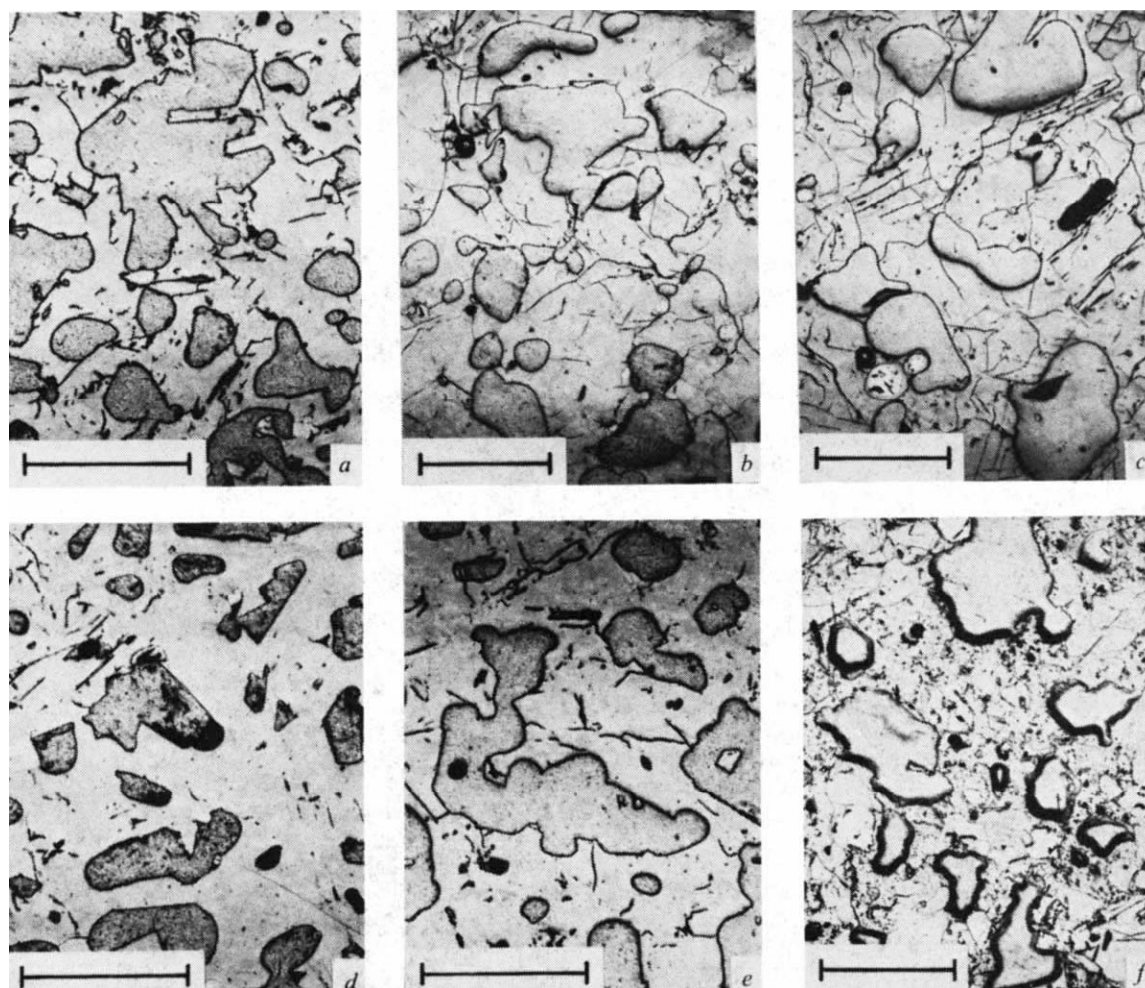


Fig. 1 Reflected light photomicrographs of mafic inclusions in some andesitic lavas, showing the large proportion of vesicles. *a*, Llao Rock dacite, Crater Lake (Oregon). *b*, Mt Hood andesite (Oregon). *c*, Glacier Peak dacite (Washington). *d*, Chaos Crag dacite, Lassen Volcanic National Park (California). *e*, Nevada Antizana dacite (Ecuador). *f*, Chicoma Mountain dacite, Jemez Mountains (New Mexico). Scale bars: *a*, *d* and *e*, 0.2 mm; and *b*, *c* and *f*, 0.5 mm. Only the vesicles in sample *f* are not filled with epoxy resulting in greater apparent relief. Density data for inclusions *a*, *d* and *f* are reported in Table 1. Transmitted light views are presented in ref. 5.

physical properties. All inclusion samples have a grain density near 2.8 g cm^{-3} , because they consist largely of plagioclase and hornblende or pyroxene. Bulk densities are very consistent within a single volcanic unit, and show only a 10% variation from an average for the four volcanoes of 2.1 g cm^{-3} . Porosity of the samples ranges from 21 to 33%. Inclusions always have a lower bulk density than their nonvesicular hosts.

Consistency in texture and density relationships suggests that vesicularity of the inclusions may be a primary feature related to their formation and/or incorporation in the host magma. If this is the case and the vesicles, on formation, occupy half or more of the volume fraction observed in the samples, then the inclusions would be buoyant within the magma reservoir. The effect of this phase change on magmatic flow could easily exceed the effect of thermal expansion by an order of magnitude.

Vesiculation during crystallization

The potential importance of the inclusions to flow within the reservoir means we should evaluate conditions in which the vesicles could be primary. Consider a hot mafic magma which is suddenly brought into contact with a large volume of cooler silicic magma, so that the two magmas are initially in thermal and chemical disequilibrium. The rate at which temperature and concentration of water change towards equilibrium values is proportional to thermal diffusivity (K) and chemical diffusivity of water ($D_{\text{H}_2\text{O}}$), respectively. As K is several orders of magnitude larger than $D_{\text{H}_2\text{O}}$ (ref. 8), mafic magma quickly reaches thermal equilibrium but not chemical equilibrium with the reservoir, and, therefore, can be regarded as a system open to extraction of heat but initially closed to extraction (or addition) of water. The response of this system to extraction of heat is crystallization. However, only the melt phase can contain

significant amounts of water. The decrease in melt fraction of this system from nearly 1.0 to ~ 0.1 ($\sim 10\%$ glass by volume observed in inclusions, recalculated vesicle-free) raises the concentration of water in the melt by an order of magnitude. Even for low initial concentrations of water in mafic magma, this enrichment will cause exsolution of a vapour phase at crustal pressures, forming a foam of reduced bulk density. Similar conclusions apply to other possible volatile components, but only water will be considered here, as it is believed to be the dominant volatile component in calc-alkaline volcanism^{9,10}.

Cooling can thus cause vesiculation, but the depth at which such a process will be significant strongly depends on volatile content of mafic magma. Bulk density of the mafic foam can be estimated for the water contents and magmatic conditions of interest, because the densities of crystals, melt and water vapour as a function of pressure and temperature, and the solubility of water in melt as a function of pressure, are known or approximately known. Analyses of mafic inclusions and closely associated mafic lavas indicate that the type of mafic magma involved is high alumina basalt or basaltic andesite^{1,11,12}. Explosive eruption style, common presence of hornblende phenocrysts, and inferred water content of glass inclusions in phenocrysts indicate that such magmas are volatile-rich, with an estimated water content^{13,14} of 3–5 wt% (although some estimates are lower¹⁵). Figure 3 contrasts bulk density of high alumina basaltic magma containing 3 wt% H_2O during crystallization at 1.5 kbar with the bulk density of similar anhydrous magma. Both systems initially become more dense in response to growth of crystals. However, once vapour saturation occurs in the wet system, continued crystallization causes bubble growth which dramatically lowers bulk density of the magma. Figure 4 shows bulk density of foam formed from basalts with various water contents as a function of pressure at 90% crystallization, the degree of

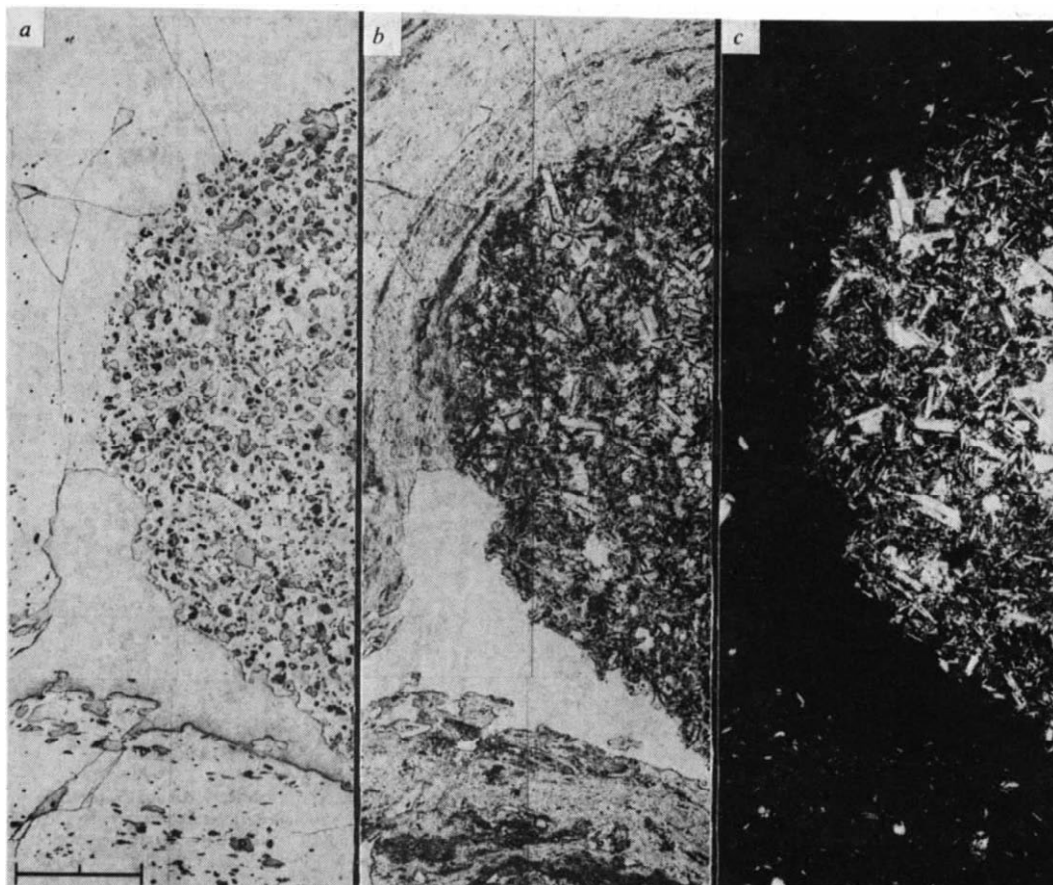


Fig. 2 Photomosaics of mafic inclusion in rhyodacitic lava from the Glass Mountain flow, Medicine Lake Highland Volcano, California. The inclusion is predominantly plagioclase and pyroxene with lesser amounts of glass, olivine, and oxides. The host lava is rhyolitic glass with varying amounts of basaltic debris. Scale bar, 1.0 mm. *a*, Reflected light, reveals the abundance of 0.1 mm-diameter vesicles in the inclusion and their scarcity in the host lava. A large vesicle is present at the lava/inclusion interface in the lower portion of *a*. *b*, Transmitted light, shows the mafic character of the inclusion in contrast to the host. Plagioclase crystals within the inclusion and the large vesicle at the interface are apparent. *c*, Taken with transmitted light and crossed polars, shows the crystal-rich character of the inclusion. Density data for these materials are presented in Table 1.

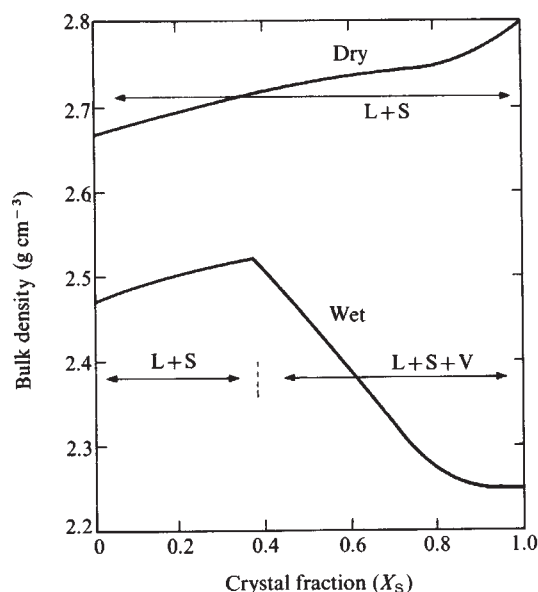
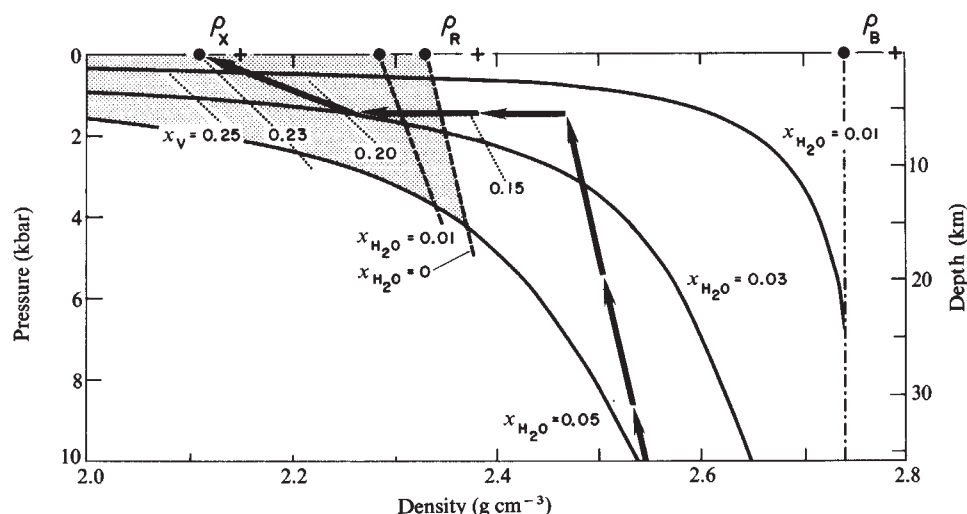


Fig. 3 Calculated bulk density plotted against degree of crystallization for wet and dry high alumina basaltic magma. The wet curve is for basalt with 3 wt% water at 1.5 kbar. Crystal fraction (X_s) is the ratio of crystals to crystals plus melt, by volume: $X_s = 0$ at the liquidus and $X_s = 1$ at the solidus. When mafic magma enters a cooler silicic reservoir, X_s increases with time from near zero until thermal equilibrium is reached near 1. To calculate bulk density as a function of X_s , the density of the crystal fraction was assumed to be constant at 2.80 g cm^{-3} , based on measured values of grain density of inclusions (Table 1) and allowance for thermal expansion¹⁶. Melt composition was calculated assuming constant crystal fraction composition such that the anhydrous melt composition is rhyolitic⁶ at $X_s = 0.9$, using an average high alumina basaltic composition¹¹ for the bulk system. Temperature was assumed to decrease linearly with increasing crystallization (X_s) so that $T = 1,200^\circ\text{C}$ at $X_s = 0$ and $T = 900^\circ\text{C}$ at $X_s = 0.9$. Melt density was then calculated from melt composition at appropriate temperature and pressure¹⁷. Vapour fraction of the system was calculated from solubility of water in melt¹⁸ and density of the vapour phase¹⁹. The calculated bulk density at low to moderate crystallization has uncertainties in assumed conditions, compositions and density of hydrous melt. However, the form of the curve and the bulk density near complete crystallization, which depends mostly on the measured grain density (Table 1) and vapour density, are not significantly affected by these uncertainties. Higher water content or lower pressure result in vapour saturation at lower crystal fraction and greater reduction in bulk density at advanced crystallization.

crystallization observed in the inclusions. Within the range of water contents estimated for high alumina basalts, a significant density decrease accompanies crystallization at mid- to upper-crustal pressures.

Fig. 4 Calculated density plotted against pressure (depth) for mafic foam ($X_s = 0.9$) of various water contents, and measured densities for Medicine Lake lava. ρ_X and ρ_B are bulk and grain density of mafic foam, respectively, and ρ_R denotes density of reservoir magma. X_{H_2O} is weight fraction of water in magma and X_V is volume fraction of vapour in magma (that is, porosity). Density of reservoir magma (ρ_R) with 0 and 1 wt% water¹¹ at $T = 900^\circ\text{C}$ was calculated from measured density of lava using data for thermal expansion and compressibility of silicate melt²⁰. The possible region for the Medicine Lake volcano magma chamber is shaded, constrained by the conditions that $\rho_X < \rho_R$ and $X_{H_2O} < 0.05$. A possible path for magma feeding the chamber is indicated by arrows. —, ρ_X at constant X_{H_2O} ; ···, ρ_X at constant X_V ; ---, ρ_R at constant X_{H_2O} ; +, measured ρ , $T = 20^\circ\text{C}$; ●, calculated ρ , $T = 900^\circ\text{C}$.



Flotation of foam

It has been argued that the arrangements of vents in andesitic and bimodal volcanic fields, as well as the history and petrology of these fields, indicate episodic replenishment of crustal magma systems by mafic magma from the mantle^{5,21-30}. Such magma should initially fill the base of the crustal reservoir, resulting in a stratified system of two fluids. Direct evidence of such stratification, which has been suggested for other types of magma reservoirs³¹, is provided by the compositional zonation of some large tephra sheets, as at Crater Lake^{12,32}. The stratification of two fluids is stable if the denser layer is on the bottom. However, Rayleigh-Taylor instability results if the denser layer is on top³³. Thus, if conditions (volatile content, pressure) permit formation of a mafic foam less dense than the reservoir magma, the interface becomes unstable in Rayleigh-Taylor fashion and blobs of foam will rise into the reservoir magma. Large-scale convection in the reservoir magma driven by heating^{8,26,34-36} of, and addition of low density material to, its base would contribute to dispersal of the mafic foam.

From the time that mafic magma enters the reservoir, a foam layer will form at the interface and thicken downwards. The rate of thickening by foam generation is controlled by the thickness of the layer and the rate of removal of foam is controlled by the rise of blobs from the interface (Fig. 5). Inclusion textures indicate that the foam has sufficient crystal content to be cohesive, so it is likely that separation of blobs from underlying material occurs at the base of the foam layer. Furthermore, if the blobs were derived from disaggregation of a large mass many diameters thicker than the inclusions, then a large range of textures representing a large range of cooling rates would be observed. Thus, layer thickness may be comparable with blob diameter. These considerations suggest a dynamic process, in which foam is continually added to a thin layer by downward propagation of a cooling wave and removed from the layer by flotation. For this to be the case, thickening of the foam layer by conduction must approximately balance thinning of the layer by flotation of blobs. A 20-cm layer, the maximum common diameter of inclusions, would thicken at a rate of the order of $10^{-4} \text{ cm s}^{-1}$ (Fig. 5), close to the rate at which material in the form of blobs 20 cm in diameter and 2.1 g cm^{-3} in density ($\Delta\rho = 0.3 \text{ g cm}^{-3}$) would be removed by flotation in 10^8 P reservoir magma. Cooling rates for the mafic magma would be of the order of 10°C h^{-1} . Basalt crystallization experiments have produced textures similar to those of the inclusions, characterized by acicular and skeletal plagioclase, at comparable cooling rates^{39,40}. Thus, both the size and texture of the inclusions seem to be consistent with this hypothesized origin.

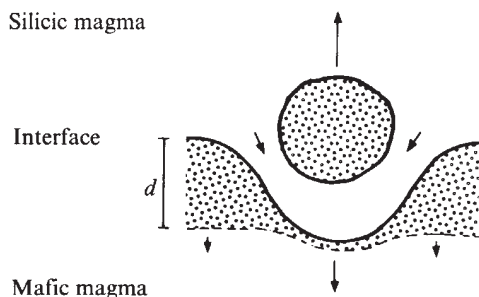


Fig. 5 Formation of mafic foam (patterned) in the region of the interface between mafic and silicic magma. A cooling wave advances downwards into the mafic magma while foam is removed from the interface by flotation. Within the mafic magma there is an upward transition from mafic liquid below the cooling wave, through less mafic liquid + crystals, to the foam layer, consisting of silicic liquid + crystals + vapour, below the interface. Within the reservoir magma, resorption or reaction of phenocrysts occurs near the interface. The base of the foam layer will be near the isotherm representing $1/2\Delta T$. If the interface is kept approximately isothermal by flow of silicic magma (upper limit case for rate of foam formation), then the thickness of the foam layer is $(Kt)^{1/2}$ and the rate at which it thickens is $K/2d$ (ref. 37). Using thermal conductivity data³⁸ and including the effect of crystallization gives $K = 3 \times 10^{-3} \text{ cm}^2 \text{ s}^{-1}$ so that a 20-cm layer thickens at $10^{-4} \text{ cm s}^{-1}$. When the foam layer has grown to this thickness, removal of foam by flotation approximately balances growth of the layer by conduction.

The field of Rayleigh–Taylor instability and hence mixing of mafic material into a silicic reservoir can be defined in terms of water content of basaltic magma and pressure. Much more water is required for flotation of foam at higher pressure because both density of water vapour and solubility of water in melt increase with pressure. Figure 4 shows the possible conditions for mixing in the magma reservoir from which the Medicine Lake dacite of Table 1 erupted. The inference that the inclusions floated constrains depth of the reservoir to ≤ 15 km for basalt with 5 wt% water, or 7 km for basalt with 3 wt% water. Data from the other volcanoes studied give similar results. Although volatile content of basalt is a major unknown, the flotation process is clearly limited to magma reservoirs in the upper crust. This conclusion is consistent with the association of foam-bearing lavas with calderas or caldera-like structures, and with the occurrence of analogous intrusive rocks as large, shallowly emplaced plutons.

A possible path of mafic magma feeding such a reservoir is also shown in Fig. 4. The basalt ascends through the crust, slowly becoming less dense with decreasing pressure. As it enters the magma reservoir at 1.5 kbar it is still water unsaturated. As it fills the base of the reservoir, heat is quickly transferred to silicic magma across the large area of the interface so that the mafic magma undergoes rapid crystallization (following the curve in Fig. 3) forming a foam with a bulk density of 2.25 g cm^{-3} , and

porosity of 0.19. If this foam remains rigid but leaks after formation, it will follow a constant porosity curve as it flows upwards. If it maintains an equilibrium pore pressure and does not leak, it will follow a constant water curve and blow up during ascent. Obviously, the foam does expand infinitely. As with silicic tephra⁴¹, growth of vesicles is retarded by increasing viscosity of the residual melt and interference from neighbouring crystals and vesicles. That the inclusions do leak water after their formation is suggested by the common presence of large vesicles at the inclusion/lava interface, as shown in Fig. 2.

Petrological implications

An important aspect of the problem of silicic magma reservoirs open to replenishment is the stability of the stratification induced by replenishment. In the stable case, any mixing is limited to the very slow process of diffusion across the interface between the two separately convecting magmas. Magma in the reservoir is heated by injection of mafic magma and may, therefore, grow by crustal melting, but it evolves in a manner chemically independent of the primitive magma feeding the chamber. In the unstable case, mixing occurs by rapid transport of mafic material across the mafic/silicic interface and into the convecting reservoir magma. The composition of magma in the reservoir reflects the combined effects of mixing, which makes the magma more mafic, and crustal melting and/or differentiation, which make it more silicic. I suggest that bimodal volcanism represents the stable case, while an important stage in the evolution of andesitic volcanoes and granodioritic batholiths represents the unstable case.

Although these conclusions are based primarily on investigation of certain volcanic rocks of the andesite family, their most important application in terms of volume is to granodioritic batholiths, in which mafic inclusions are a nearly ubiquitous feature. Recent work suggests that most of these inclusions are the plutonic equivalent of the extrusive materials described here^{42,43}. If so, these large magma bodies may initially evolve as bimodal systems in the deeper crust, with formation and accumulation of granitic crustal melt in response to basaltic volcanism^{44–46}. Subsequent rise of granitic magma bodies into the upper crust within regions of upward flux of wet basaltic magma would result in mixing driven by vapour exsolution, with generation of granodioritic bulk compositions. Direct exsolution of a vapour phase from basaltic magma within upper crustal reservoirs may also play a part in formation of ore deposits, which are sometimes associated with granodioritic intrusives⁴⁷.

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Specific high affinity cell membrane receptors for biologically active phorbol and ingenol esters

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A variety of normal and transformed avian and mammalian cells and tissues contain high affinity specific receptors on their membranes which interact with biologically active phorbol and ingenol esters in a reversible and saturable manner. The binding of labelled phorbol-12,13-dibutyrate (PDBu) to live or glutaraldehyde-fixed cells is dose-, time- and temperature-dependent. Those phorbol or ingenol esters which stimulate cell growth in culture and have tumour-promoting activity in vivo inhibit the binding of labelled PDBu, while the biologically inactive derivatives fail to do so. Other non-diterpene tumour-promoting agents, epidermal growth factor (EGF), retinoids and prostaglandins do not compete for the binding of labelled PDBu to its specific membrane receptors.

TUMOUR promoters are compounds which are themselves non-carcinogenic but which can induce tumours in animals previously treated with a suboptimal dose of certain chemical carcinogens¹⁻⁵. Most of the experimental work on tumour promotion has been carried out with phorbol esters, especially 12-*O*-tetradecanoylphorbol esters (TPA), initially isolated from croton oil derived from the seed of the plant *Croton tiglium*^{4,6}. TPA and other biologically active phorbol esters elicit and modulate a variety of biochemical and biological responses in mouse skin, including stimulation of macromolecular synthesis, histone phosphorylation, synthesis of phospholipids and modulation of the metabolism of polyamines and cyclic nucleotides^{1-5,7-13}. In addition, these compounds induce ultra-structural changes in and affect the differentiation of murine epidermis^{7,14}. Tumour-promoting phorbol esters also evoke pleiotypic responses in cultured cells, including the stimulation of macromolecular synthesis and cell proliferation, induction of plasminogen activator and ornithine decarboxylase, loss of surface-associated fibronectin, alterations in the metabolism of cyclic nucleotides and polyamines, stimulation of prostaglandin synthesis, either the inhibition or stimulation of differentiation, and alterations in cell morphology and cell permeability, and elevation in the level of $[Na^+K^+]ATPase$ activity^{1-5,15-23}.

Several biochemical and biological studies provide evidence that the initial site of action of tumour-promoting phorbol esters may be the membrane of target cells^{3-5,21,23-26}. The tumour-promoting phorbol esters have been found to modulate the interaction between epidermal growth factor (EGF) and its membrane receptors in a variety of cells in culture²⁷⁻²⁹. The pleiotypic effects of TPA and related tumour promoters *in vivo* as well as *in vitro* seem to mimic the several actions of growth-stimulating polypeptide hormones such as EGF³⁰ and sarcoma growth factor (SGF)³¹. However, the effect, though rapid in modulating the EGF receptors, is indirect as it cannot be shown using low temperatures²⁸ and/or fixed cells or in isolated cell membranes (unpublished results). This would suggest that TPA produces its membrane effects through an interaction distinct from the EGF receptor interaction.

³H-TPA has been reported to bind to HeLa cells in a non-saturable, noncompetitive and nonspecific fashion³². We find that ³H-TPA binds to a variety of fibroblastic and epithelioid cells in saturable and reversible manner but these binding sites for TPA seem to be numerous (30×10^6 per cell) and of low affinity ($K_d = 10^{-7}$ M). We have used ³H-PDBu to characterize the binding sites of phorbol and ingenol esters. PDBu is a phorbol ester which is highly active *in vivo* and *in vitro* but is

comparatively less lipophilic than TPA^{4,33}. Thus, PDBu would partition in membrane lipids much less than TPA and lead to less nonspecific binding. Recently, ³H-PDBu binding to a particulate fraction of chicken embryonic fibroblasts has been reported³⁴.

Binding of PDBu to Cells

Figure 1 describes the binding of ³H-PDBu (19.7 ng ml⁻¹, specific activity 6.4 Ci mmol⁻¹, Life System Co., Newton, Massachusetts) to live and fixed mink lung cells (CCL 64)³⁵ and BALB/3T3 clone A31 cells (CLL 163)³⁶ at 23 °C, and the competition of binding of labelled PDBu to cells with unlabelled PDBu. The live and the fixed mink cells bound 2,155 and 1,987 c.p.m., respectively, whereas live and fixed A31 cells bound 3,605 and 3,419 c.p.m., respectively, of ³H-PDBu per 10⁶ cells in the absence of unlabelled PDBu. As the concentration of unlabelled PDBu was increased, the binding of ³H-PDBu to both types of cells decreased. Figure 1 shows that viable and glutaraldehyde-fixed mink cells required 30–35 ng ml⁻¹ of PDBu for 50% inhibition of binding; the A31 showed ID₅₀s of 65–70 ng ml⁻¹ and, again, no difference between viable and fixed cells.

Figure 2a shows the extent of binding of ³H-PDBu to mink lung cells at various temperatures, as a function of incubation time. The binding increased linearly for 15 min at 4 °C, whereas binding at 23 °C and 37 °C was linear for only a few min. Maximal binding was achieved within 7.5 min at 37 °C and started to decrease with increasing incubation time. The saturation of the binding of labelled PDBu to cells at 23 or 4 °C was observed between 20–40 and 90–120 min, respectively. When ³H-PDBu-labelled mink lung cells (23 °C, 30-min incubation) containing bound ³H-PDBu were incubated in only binding buffer at 4, 23 or 37 °C, bound radioactivity dissociated from cells and was released into the medium in a time- and temperature-dependent manner (Fig. 2b).

Optimum binding of PDBu was achieved at pH 6.7. The binding was found to be linear up to 12×10^6 cells ml⁻¹ (23 °C, 20-min incubation). It was not significantly affected by hydroxyurea (10 mM), actinomycin D (10 µg ml⁻¹), cycloheximide (10 µg ml⁻¹) or sodium fluoride (1 mM). Thus, it seems that PDBu binding to its receptors does not require DNA, RNA or protein synthesis, or metabolic energy. We did not find any absolute requirements of metal ions for PDBu binding to cells; however, metal chelating agents (EDTA and EGTA) at 5 mM concentration reduced the binding to approximately 50%. Mg²⁺, Co²⁺, Na⁺ and K⁺ did not significantly affect the binding

although Mn^{2+} , Ca^{2+} and Zn^{2+} significantly stimulated the binding of 3H -PDBu to mink cells.

Sub-cellular distribution of bound 3H -PDBu to mink cells ($4^\circ C$ for 30 min or $23^\circ C$ for 10 min) revealed that approximately 80% of labelled PDBu bound to the membranous fraction. This result and the comparable binding of 3H -PDBu to fixed or live cells suggest that the binding sites are located on the plasma membranes of cells.

Wide distribution of PDBu binding sites in cell cultures and various murine tissues

Table 1 shows the specific binding of 3H -PDBu to various normal cell lines from different species in culture and murine cell lines transformed by RNA tumour viruses, DNA tumour viruses, SV40 and polyoma or by chemical carcinogens, benzo-pyrene (BP) and methylcholanthrene (MC). PDBu bound to murine (normal or transformed), rat, cat, mink, hamster, rabbit, bat, dog, monkey, human and chicken cells. The transformed murine cells were found to bind more PDBu than their normal counterparts. 3H -PDBu also bound to Kirsten sarcoma virus transformed murine cells as well as to Swiss/3T3 clone NR-6; both cell lines lack EGF receptors^{37,38}. The specific binding sites are also present in murine brain, spleen, thymus, lung, skin, kidney, heart, thigh muscle, liver and intestine in decreasing order. The murine brain which lacks EGF receptors and spleen

has an exceptionally high number of receptors for PDBu. Neither human nor mouse erythrocyte bound detectable amounts of 3H -PDBu. Thus, the PDBu binding sites are widely distributed and are distinct from EGF binding sites.

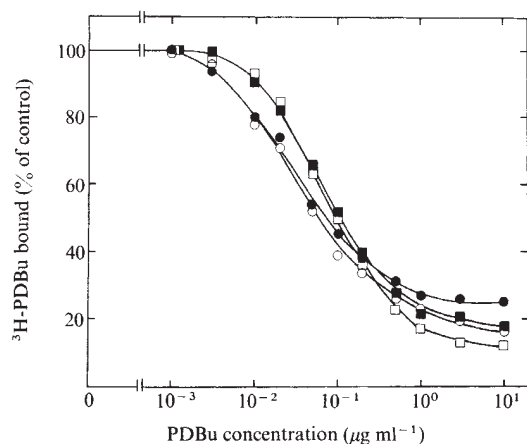


Fig. 1 Binding of 3H -PDBu to live and fixed cells and inhibition of 3H -PDBu binding by unlabelled PDBu. The cells were grown in 850 cm² roller culture bottles (RCB) in Dulbecco's minimum essential medium (DMEM) containing 10% calf serum (CS). Approximately 70% confluent cells were used for the binding assays. The medium was aspirated from bottles, cells were washed twice with 50 ml of binding buffer (BB) consisting of DMEM containing bovine serum albumin (BSA, 1 mg ml⁻¹) and *N,N*-bis-(2-hydroxyethyl)-2-amino-ethane-sulphonic acid (BES, 5 mM), adjusted to pH 6.8. The cells were scraped in 25 ml of BB per RCB and transferred to plastic tubes and pelleted in a refrigerated centrifuge. The cell pellets were suspended in BB and an aliquot used to determine cell number in a Coulter counter. The binding assays were performed in duplicate in 12 × 75 mm disposable glass culture tubes (Kimax). The binding mixture contained 5 ng 3H -PDBu ($\sim 4 \times 10^4$ c.p.m.), 0–5 μ g of unlabelled PDBu, 0.5% final concentration of dimethyl sulphoxide (DMSO) and 1×10^6 cells in a total volume of 0.25 ml BB. After incubation for 30 min at $23^\circ C$, 4 ml cold BB was added to each tube, the contents mixed and tubes were centrifuged for 4 min at $4^\circ C$ at 1,000g to pellet the cells. The supernatant solution was carefully drained off, the pellet was suspended in 4 ml of cold BB and transferred to other tubes. Tubes were again centrifuged as earlier, supernatant solution was removed and cell pellets solubilized in 0.7 ml of lysing buffer (0.01 M Tris-HCl, pH 7.4, containing 0.5% SDS and 1 mM EDTA). The mixture was transferred to counting vials and 10 ml of Optigel (Melay Laboratories) was added to each vial. The vials were vigorously shaken and radioactivity determined using a Beckman beta counter. For binding assays with fixed cells, the medium was aspirated, cells were washed twice with cold PBS and scraped from RCB and transferred to plastic tubes and pelleted. The pellet was suspended in 2% glutaraldehyde, pH 7.4 (1 ml per 10^6 cells) for 2 h at $4^\circ C$. After this period, cells were pelleted and washed twice with cold PBS and the final pellet was suspended in BB. The binding with fixed cells was performed as described for the live cells. $\circ$, Live mink lung cells; $\bullet$, fixed mink lung cells; $\square$, BALB/3T3-A31 cells; $\blacksquare$, fixed BALB/3T3-A31 cells.

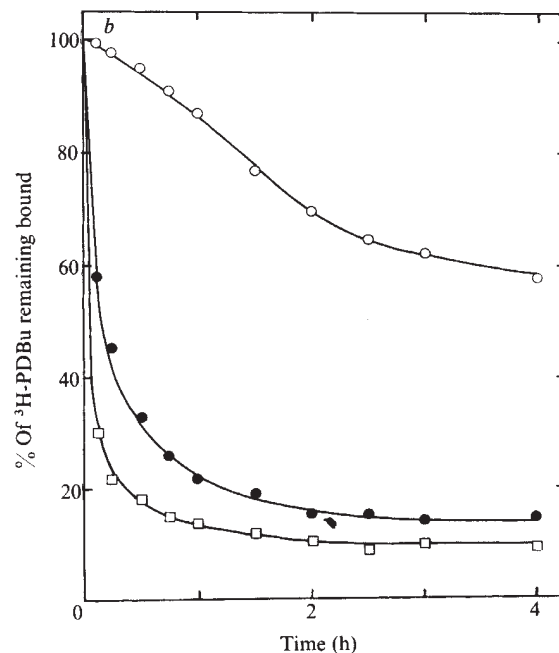
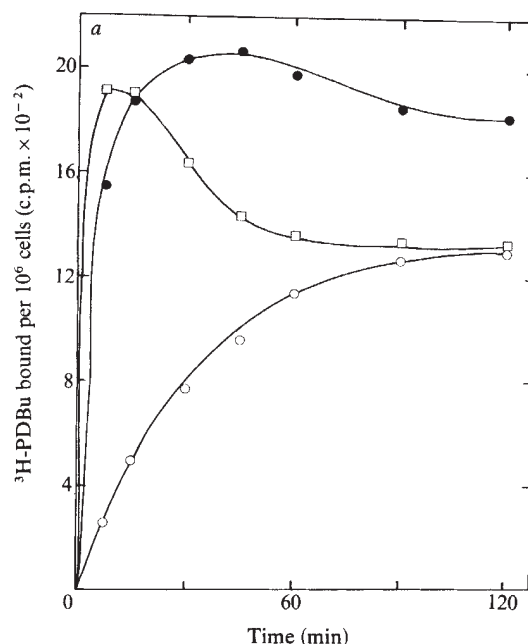


Fig. 2 *a*, Effect of incubation time and temperature on 3H -PDBu binding to mink lung cells. The binding assays were performed in duplicate as in Fig. 1 legend, in the presence or absence of 20μ g ml⁻¹ unlabelled PDBu. The radioactivity bound to cells in the presence of 20μ g ml⁻¹ unlabelled PDBu was considered to be nonspecific binding and all data were corrected accordingly. $\circ$, $4^\circ C$; $\bullet$, $23^\circ C$; $\square$, $37^\circ C$. *b*, Dissociation of bound 3H -PDBu from mink lung cells. Binding of 3H -PDBu (30 min at $23^\circ C$) was performed as described in Fig. 2*a* legend. After the final washing, cells were suspended in BB (0.25 ml per 10^6 cells) and duplicate mixtures were incubated at each indicated temperature for each indicated time. After incubation, 4 ml of cold BB was added to each tube, cells were mixed and pelleted as described in Fig. 1 legend. The supernatant solutions were removed, pellets suspended in the lysing buffer and counted as in Fig. 1 legend. The nonspecific binding was corrected as in *a*. $\circ$, $4^\circ C$; $\bullet$, $23^\circ C$; $\square$, $37^\circ C$. The released labelled material was analysed by HPLC and was found to be PDBu.

Table 1 Binding of ^3H -PDBu to cells of various species

Species	Cells	^3H binding (c.p.m. per 10^6 cells)
Chicken	Embryo fibroblasts	4,273
Mouse	BALB/3T3-A31	3,506
	Murine sarcoma virus transformed A31	4,995
	Benzo[a]pyrene transformed A31	5,790
	SV40 transformed A31	5,505
	Methylcholanthrene transformed A31	7,983
	NIH/3T3 Cl 142	3,285
	Polyoma virus transformed NIH/3T3 Cl 4A	6,729
	Swiss/3T3 Cl 8	3,635
	Swiss/3T3-NR6 (no EGF receptors)	3,342
	Swiss/3T3 Cl LI (preadipocytes)	1,560
	129/J F9 (teratocarcinoma)	487
	Swiss mouse erythrocytes (fresh)	6
Rat	NRK (fibroblasts) clone 49F	2,824
	NRK (epithelial) VB-4	1,928
Hamster	Ovary CHO	3,357
Mink	Lung (epithelial) Mv1Lu (CCL 64)	2,074
Bat	Lung (fibroblastic) Tb1Lu (CCL 88)	2,498
Cat	Embryo (FEC) Cl 60	3,362
Dog	Thymus cf2Th	1,626
Monkey	Rhesus RBS	1,407
Human	Fibroblast (CCL 1553)	4,360
	Cervical carcinoma HeLaS3	864
	Epidermal carcinoma of vulva A431	2,250
	Acute myelogenous leukaemia HL60	2,114
	Erythrocytes	12

The binding assays were performed as in Fig. 1 legend. The nonspecific binding was corrected as described in Fig. 2 legend.

Saturability of PDBu Binding

The effect of PDBu concentration on PDBu binding to mink lung cells and murine BALB/3T3-A31 cells is shown in Fig. 3. PDBu binding was found to be linear up to a PDBu concentration of about 5 ng ml^{-1} for both cell types. As the concentration of PDBu was increased beyond 5 ng ml^{-1} , the binding of PDBu to cells gradually began to deviate from linearity and started to level off. PDBu binding sites were almost saturated at a PDBu concentration of 200 ng ml^{-1} (Fig. 3, insets). Figure 3 also includes Scatchard plots of PDBu binding to mink lung cells and to BALB/3T3 cells. These data produced almost linear plots for both cell types. At saturating concentration of PDBu, approximately 2.0×10^5 molecules bound to mink lung cells and 5.1×10^5 molecules to BALB/3T3 cells. The apparent K_d values were calculated to be $1.3 \times 10^{-9} \text{ M}$ for mink lung cells (Fig. 3a) and $0.9 \times 10^{-9} \text{ M}$ for BALB/3T3 cells (Fig. 3b). The apparent K_d value for EGF interaction to its receptors on both cell types has been reported to be $0.5 \times 10^{-9} \text{ M}$ (ref. 28). Thus, the affinity of PDBu for its receptors is in the same range as that of EGF receptors for its ligands and other ligands for their receptors²⁸.

Relationships between structure of phorbol and ingenol derivatives and binding to mink lung cells

There are now available several natural and synthetic analogues of phorbol and ingenol with various degrees of promoting activity in the two-stage tumorigenesis model¹⁻⁵. We studied their effects on inhibiting the binding of ^3H -PDBu to mink lung cells (Fig. 4 and Table 2). TPA was the most potent competitor of PDBu binding among the phorbol, ingenol and mezerein derivatives tested. The dose required for 50% inhibition of PDBu binding to cells for TPA, PDBu, phorbol-12,13-didecanoate (PDD), phorbol-12-13-dibenzoate (PDB), 12-deoxyphorbol-13-tetradecanoate (DPTD), ingenol-13-hexadecanoate (IHD), mezerein (MZ), phorbol-12,13-diacetate (PDA) and ingenol 3,5,20-triacetate (ITA) were $9.6 \times 10^{-9} \text{ M}$, $9.9 \times 10^{-8} \text{ M}$, $13.4 \times 10^{-8} \text{ M}$, $18.2 \times 10^{-8} \text{ M}$, $2.4 \times 10^{-8} \text{ M}$, $5.8 \times 10^{-8} \text{ M}$, $7.2 \times 10^{-8} \text{ M}$, $6.4 \times 10^{-6} \text{ M}$ and $9.8 \times 10^{-6} \text{ M}$, respectively; 4 α -PDD, 4-O methyl-TPA (MeTPA), phorbol and ingenol had negligible effects on PDBu binding (Fig. 4a, b). The relative potency of these agents in competing EGF binding was: TPA > DPTD > IHD > MZ > PDBu > PDD > PDB > PDA > ITA > Me-TPA > 4 α -PDD > phorbol > ingenol (Table 2). The inhibition of PDBu binding to its receptors by different phorbol and ingenol derivatives correlated very well with their tumour-promoting activity. Table 2 also summarizes the dose required for 50% inhibition of EGF binding for various phorbol derivatives (data taken from ref. 28) and for ingenol derivatives and MZ (unpublished data). 2 ng ml^{-1} of ^{125}I -EGF and 19.7 ng ml^{-1} of ^3H -PDBu were used in these binding experiments. Table 2 shows that ID values for PDBu binding and for the indirect effect on EGF binding, as well as calculated K_i values for PDBu binding and EGF binding for the various agents correlated very well with each other and with their tumour-promoting potential. The relative values (ID_{50}/K_i) for various agents were found to be almost similar.

Lack of competition of PDBu binding by non-diterpene ester tumour promoters and by certain other compounds

Non-diterpene ester tumour promoting agents such as phenol, iodoacetic acid, iodoacetamide, bile acids, barbiturate, oleate, laurate, limonene, canthradin, anthradin, saccharin, or cyclamate¹⁻⁵, did not affect the binding of ^3H -PDBu to its receptors even up to a concentration of $10 \mu\text{g ml}^{-1}$. However, anthralin, another tumour promoter, actually enhanced the binding of PDBu to mink lung cells (Table 3). Thus, it seems that non-diterpene ester tumour promoting agents do not exert their biological effect through these membrane receptors. Retinoic

Table 2 Correlation between the potency of phorbol and ingenol derivatives for promoting skin tumours and their ability to inhibit the binding of ^3H -PDBu or ^{125}I -EGF to cells

Compounds	Dose required for 50% inhibition (ng ml^{-1})		K_i values (M)†		Tumour-promoting activity ¹⁻⁵
	PDBu binding to its receptor*	EGF binding to its receptor†	PDBu binding to its receptors	EGF binding to its receptors	
12-O-tetradecanoylphorbol-13-acetate (TPA)	5.9	1.8	2.3×10^{-10}	1.8×10^{-9}	+++
Phorbol-12,13-dibutyrate (PDBu)	50.2	32.8	2.4×10^{-9}	3.9×10^{-8}	++
Phorbol-12,13-didecanoate (PDD)	83.5	53.8	3.2×10^{-9}	5.2×10^{-8}	++
Phorbol-12,13-dibenzoate (PDB)	104.0	105.0	4.4×10^{-9}	1.1×10^{-7}	+
4 α -Phorbol-12,13-didecanoate (4 α -PDD)	>100,000	>10,000	$>1.6 \times 10^{-4}$	$>9.6 \times 10^{-6}$	Non-promoter
Phorbol-12,13-diacetate (PDA)	3,100	>10,000	$>1.5 \times 10^{-4}$	$>12.4 \times 10^{-6}$	Non-promoter
4-O-methyl-TPA (Me-TPA)	>100,000	>10,000	$>1.7 \times 10^{-4}$	$>9.5 \times 10^{-6}$	Non-promoter
Phorbol	>100,000	>10,000	$>2.7 \times 10^{-4}$	$>16.5 \times 10^{-6}$	Non-promoter
12-deoxyphorbol-13-tetradecanoate (DPTD)	13.8	21.1	5.7×10^{-9}	2.3×10^{-8}	++
Mezerein	42.5	9.8	1.7×10^{-9}	9.9×10^{-8}	?
Ingenol-13-hexadecanoate (IHD)	34.2	101.0	1.4×10^{-9}	10.3×10^{-8}	+
Ingenol-3,5,20-triacetate (ITA)	3,400	3,000	2.3×10^{-7}	3.6×10^{-6}	Non-promoter
Ingenol	>100,000	>10,000	$>2.8 \times 10^{-4}$	$>17.2 \times 10^{-6}$	Non-promoter

* Values derived from Fig. 4. 19.7 ng ml^{-1} of ^3H -PDBu was used for binding.

† Values for phorbol derivatives are taken from ref. 28. The values for DPTD, MZ and ingenol derivatives are from our unpublished results. 2 ng ml^{-1} of ^{125}I -EGF were used for binding.

‡ K_i values were calculated according to method of Cheng and Prusoff⁴², using the equation $K_i = \text{ID}_{50} \times 1/(1 + L/K)$, where L and K denote concentration and affinity of labelled probe.

acid, dexamethasone, prostaglandins and disulfiram are reported to inhibit tumour promotion¹⁻⁵. None of these reagents significantly modulated the binding of PDBu to mink lung cells (Table 3). These results suggest that these reagents inhibit tumour promotion by acting at some site other than the receptors for phorbol or ingenol esters. In addition, cholera toxin, diphtheria toxin, oxytocin, vasopressin, gramicidin, monesin, melittin, digitonin, filipin, amphotricin, kanacidin, ganglioside, nystatin, cholesterol and lysophosphocholine, up to a concentration of $10 \mu\text{g ml}^{-1}$, did not significantly affect the binding of labelled PDBu to its receptors.

The binding of PDBu to mink cells is not inhibited by EGF even up to a concentration of $10 \mu\text{g ml}^{-1}$ (Table 3). Although biologically active diterpene esters efficiently modulate EGF

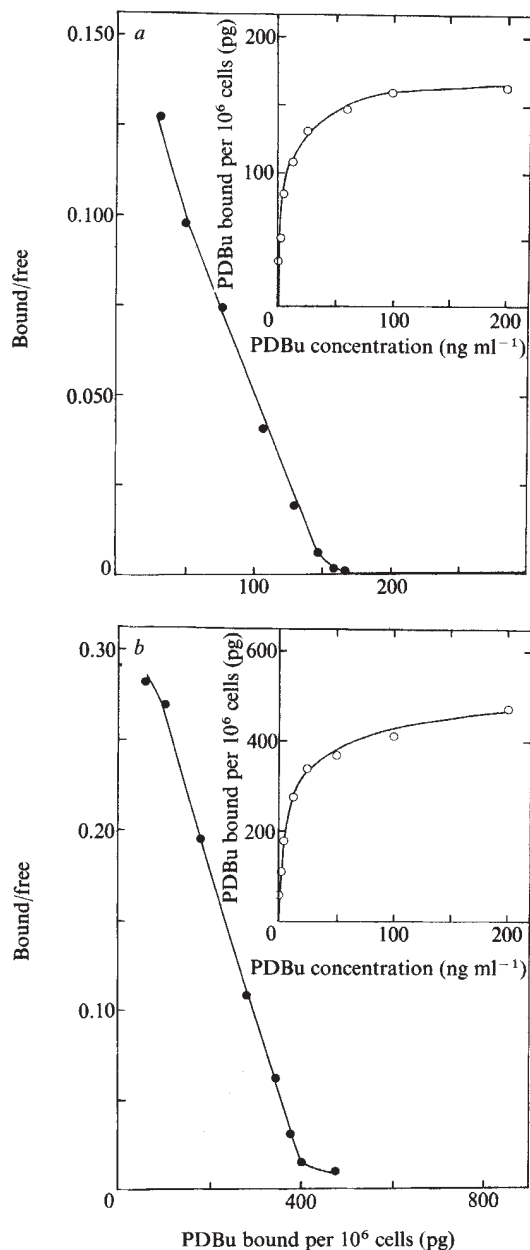


Fig. 3 *a*, Effects of PDBu concentration on binding to mink lung cells and Scatchard plot. The binding assays were performed as in Fig. 1 legend. The indicated concentrations of ³H-PDBu were used. The nonspecific binding was corrected as in Fig. 2. ○, PDBu binding as a function of PDBu concentration; ●, Scatchard plot of the data in inset. *b*, Effect of PDBu concentration on PDBu binding to BALB/3T3-A31 cells and Scatchard plot. Experiment was performed as in *a*. ○, PDBu binding as a function of PDBu concentration; ●, Scatchard plot of the data in inset.

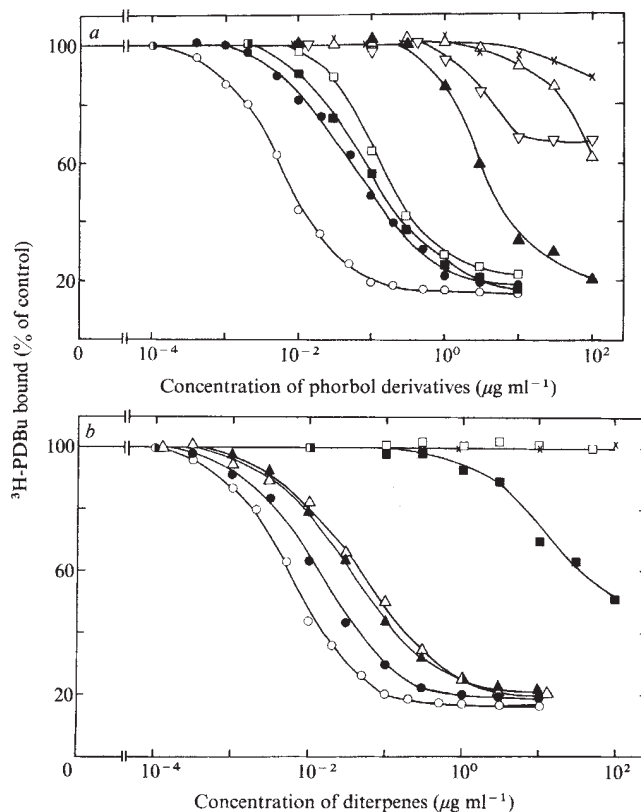


Fig. 4 *a*, Competition of the binding of ³H-PDBu to mink lung cells by various derivatives of phorbol. The binding assays were performed in the presence of ³H-PDBu (19.7 ng ml^{-1}) and the indicated concentrations of various derivatives of phorbol as described in Fig. 1 legend. ○, TPA; ●, PDBu; □, PDB; ■, PDD; △, 4 α -PDD; ▲, PDA; ▽, Me-TPA; ×, phorbol. *b*, Competition of the binding of ³H-PDBu to mink lung cells by various derivatives of ingenol, mezerein, DPTD and retinoic acid. Experiment was performed as in *a*. ○, TPA; ●, DPTD; △, MZ; ▲, IHD; □, ingenol; ■, ITA; ×, retinoic acid.

Table 3 Effect of tumour promoters and other compounds on the binding of ³H-PDBu to mink lung cells

Compounds	Concentration ($\mu\text{g ml}^{-1}$)	Binding (c.p.m. per 10^6 cells)	Inhibition of binding (%)
None	—	2,049	0
TPA	10	62	97
Phorbol	10	2,039	0
Phenol	10	2,018	2
Iodoacetic acid	10	1,967	4
Lithocholic acid	10	2,070	-1
Na-barbituate	10	2,002	2
Na-oleate	10	2,072	-1
Limonene	10	2,011	2
Anthradin	10	2,004	2
Anthrakin	1	2,449	-20
Anthrakin	5	3,133	-53
Anthrakin	10	4,180	-104
Saccharin	10	2,041	0
Cyclamate	10	2,065	1
Vitamin K ₃	10	2,079	-1
Disulfiram	10	1,997	3
Retinoic acid	50	2,088	-2
EGF	10	2,075	-1
Dexamethasone	10	2,045	1
Insulin	50	2,155	-5
Prostaglandin E ₁	50	2,106	-3
Prostaglandin F ₂	50	1,905	-7

The binding assays were performed as in Fig. 1 legend. The nonspecific binding was corrected as in Fig. 2 legend.

receptor interaction in this cell²⁸, PDBu binds to cells lacking EGF receptors (Table 1). The binding of PDBu to cells does not correlate with their EGF receptor numbers. These experiments clearly demonstrate that membrane receptors which specifically interact with phorbol or ingenol diterpene esters are structurally and functionally distinct from EGF membrane receptors.

Discussion

These experiments demonstrate the existence of high affinity sites on avian, mammalian and primate cells in culture which specifically interact with biologically active phorbol and ingenol derivatives but not with biologically inert analogues of these diterpenes. PDBu binding to its receptors exhibits specificity, saturability, and reversibility. PDBu-binding sites in cell membranes are different from EGF membrane receptors as PDBu also binds to cells which lack EGF receptors. In contrast to the modulation of EGF binding to its receptors by biologically active phorbol or ingenol esters, EGF does not influence the interaction between PDBu and its membrane receptors. These membrane receptors specifically bind biologically active phorbol and ingenol esters but not other non-diterpene ester tumour promoters. Potent inhibitors of tumour promotion such as retinoids, anti-inflammatory steroids, prostaglandins and cyclic nucleotides do not interact with these receptors. Interes-

tantly, anthralin, a non-diterpene tumour promoter, increases the binding of PDBu to mink cells in a dose-dependent manner. We do not know whether anthralin exhibits this effect by increasing the binding capacity or the binding affinity or both, or acts by some other mechanism. Whether anthralin enhances the tumour-promoting activity of PDBu and other related compounds should be investigated.

Thus, why should mammalian cells have specific receptors for biologically active phorbol and ingenol ester compounds of plant origin? TPA and certain analogues may have some structural resemblance to the endogenous growth-promoting and/or differentiation-modulating substance(s) (agonists and/or antagonists) that have specific membrane receptors. These compounds recognize and interact with the receptors, mimicking the action of the putative substance(s). Azaserine, cordycepin, curare, opiate, physostigmine, plant lectins, puromycin and tubercidin appear to exert their action by such biological mimicry³⁹⁻⁴¹. Rohrschneider and Boutwell have suggested that TPA bears some similarity to a polyunsaturated fatty acid ester (PUFA), or a metabolite of it, and might exert its effects by interacting with PUFA receptors³⁹. The binding of PDBu to fixed cells suggests that it interacts with the cellular components (receptors) located on the plasma membranes of the cells. The isolation and further characterization of PDBu receptors and putative endogenous ligand(s) should help in understanding tumour promotion.

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An actin-binding protein from *Acanthamoeba* regulates actin filament polymerization and interactions

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A protein has been purified from Acanthamoeba which, like cytochalasin B, caps the end of actin filaments normally favoured for monomer addition and inhibits the interactions of actin filaments. In addition, this 'capping' protein nucleates the polymerization of actin monomers and blocks the annealing of actin filament fragments.

REGULATION of actin filament polymerization and actin filament interactions seems to be essential for cellular motility and the maintenance of cytoplasmic consistency. Thus, cells must have mechanisms to specify actin filament number, length, stability and sites of formation, in addition to mechanisms which regulate the interactions of the filaments with each other, with membranes and with other cellular structures such as microtubules. These mechanisms are just beginning to be understood¹.

The number and length of the actin filaments could be limited partly by the concentration of polymerizable monomer and the number of filament nucleating sites. The concentration of actin monomer may be controlled by profilin², a ubiquitous small protein which binds actin monomer and inhibits polymerization^{2,3}, or by other unrecognized mechanisms which also sequester actin monomers. Even less is known about cellular nucleating mechanisms, because nucleating molecules have not previously been identified. However, electron microscopy

clearly shows that there are structures, such as the dense tips of microvilli⁴ and sperm actomeres⁵, which can specify both the site and polarity of polymerization.

A more specific mechanism for controlling filament length would be to cap one or both ends with specific proteins. In striated muscle a protein named β -actinin may block the 'pointed' end of the thin filaments⁶. (The terms 'barbed' and 'pointed' are used to specify the polarity of actin filaments which is revealed by the arrowhead-shaped complexes formed when myosin binds to the filament⁷. The barbed end is the fast, and the pointed end the slowly polymerizing end⁸.) There have been disagreements about the identification of the molecule^{6,9}, but a preparation consisting of polypeptides of molecular weights (MWs) 34,000 and 37,000 is a potent inhibitor of the annealing of sonicated actin filament fragments⁶. Non-muscle cells also have proteins which can limit actin filament length, presumably by acting on the end(s) of the filaments. In *Physarum* the protein is called plasmodium actinin and has a molecular weight of 43,000 (refs 10, 11). In macrophages the length-regulating protein, gelsolin, is calcium sensitive and has a MW of 90,000 (ref. 12).

Actin filaments can interact with each other by both cytochalasin-insensitive connections by multivalent actin-binding proteins¹³⁻¹⁶ and by cytochalasin-sensitive direct associations between filaments^{17,18}. Gels consisting of random networks of filament form when the number of cross-links is low¹⁶, whereas bundles of aligned filaments form when the number of cross-links is high¹⁵. The regulation of these interactions could, in principle, occur at three levels: the formation and breakdown of

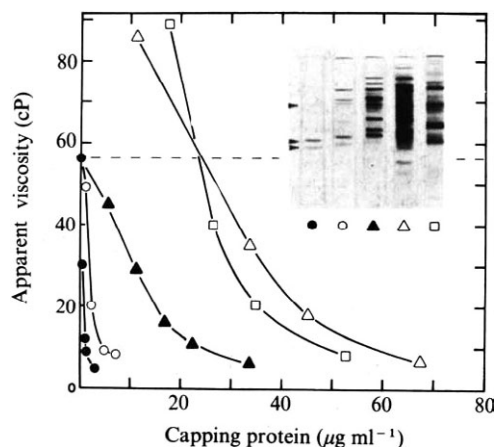


Fig. 1 Purification of the actin filament capping protein from *Acanthamoeba*. The material obtained at each step of the purification was assayed for its ability to inhibit the low-shear apparent viscosity¹⁶ of pure actin filaments and for polypeptide composition by gel electrophoresis in 7.5% polyacrylamide with 0.1% sodium dodecylsulphate¹⁶. The viscometric assay was carried out by polymerizing a mixture of 0.5 mg ml⁻¹ gel-filtered muscle actin monomers¹⁸ with the material to be tested in a buffer consisting of 10 mM imidazole, 2 mM MgCl₂, 1 mM EGTA, 1 mM ATP, pH 7.5, in a 1.2-mm inside-diameter capillary tube for 10 min at 25°C. A 0.64-mm outside-diameter stainless steel ball was then rolled down the wall of the capillary tilted at 50° from horizontal. The apparent viscosity was calculated from the ball velocity by comparison with newtonian standards¹⁶. The horizontal dashed line is the viscosity of actin filaments alone. Actomyosin supernatant (□) was obtained as described²² and the fraction precipitating between 1.5 and 2.5 M ammonium sulphate (Δ) collected and dialysed against ID buffer (20 mM imidazole, 0.5 mM dithiothreitol (DTT), pH 7.5) with 1 mM EGTA. The ammonium sulphate fraction was further purified by gel-permeation chromatography on a 4 × 95-cm column of Sephadex G-150 in the same buffer. The peak of the viscosity-inhibiting activity (▲) was then applied to a 2.5 × 19-cm column of hydroxylapatite equilibrated with ID buffer and eluted with a concave 0–250 mM phosphate gradient. The peak of viscosity-inhibiting activity (○) was dialysed against ID buffer and finally purified by chromatography on a 1.5 × 7.5-cm column of DEAE-cellulose equilibrated with ID buffer and eluted with a linear 50–300 mM KCl gradient. The purified capping protein eluted at 200 mM KCl (●) and consisted of two major polypeptides (of MWs 28,000 and 31,000) and a minor 60,000-MW polypeptide (arrowheads).

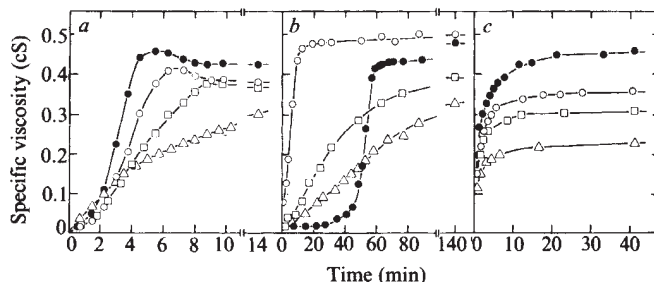


Fig. 2 Effect of filament capping protein on the polymerization kinetics of pure actin. Polymerization was monitored by measuring the high-shear viscosity in an Ostwald capillary viscometer¹⁸. *a*, Gel-filtered pure actin monomer (0.5 mg ml⁻¹) was mixed with 0 (●), 0.44 μg ml⁻¹ (○), 1.54 μg ml⁻¹ (□) or 3.08 μg ml⁻¹ (Δ) of capping protein and polymerized in 2 mM MgCl₂, 10 mM imidazole, 1 mM EGTA, 1 mM ATP, pH 7.5, at 25°C. *b*, Gel-filtered actin monomer (0.5 mg ml⁻¹) (●) was mixed with 2.2 μg ml⁻¹ capping protein (○), 30 μg ml⁻¹ actin filaments (Δ) or 2.2 μg ml⁻¹ capping protein plus 30 μg ml⁻¹ actin filaments (□) and polymerized in 20 mM KCl, 10 mM imidazole, 0.7 mM Tris, 0.07 mM ATP, 0.2 mM DTT, 0.07 mM CaCl₂, pH 7.5, at 25°C. *c*, Steady-state samples of 0.5 mg ml⁻¹ actin filaments in the buffer described in *b* were mixed with 0 (●), 2.2 μg ml⁻¹ (○), 4.4 μg ml⁻¹ (□) or 8.8 μg ml⁻¹ (Δ) of capping protein and sonicated for 15 s at setting 1 of a Branson sonicator. The recovery of viscosity was followed at 25°C.

the actin filaments¹⁹; the cross-linking of filaments by actin-binding proteins¹³⁻¹⁶; and the modulation of actin filament self-associations¹⁶. This last possibility has not been considered seriously before, but the cell might well need to regulate actin filament self-associations, because they are sufficiently strong for the viscosity of pure actin filament solutions to approach infinity at the low shear rates found in cells²⁰. The existence of an endogenous cytochalasin-like factor could explain why cytoplasm is not solid. Such a protein factor contaminates conventional muscle actin preparations^{18,21}. This unpurified factor strongly inhibits actin filament self-associations without inhibiting polymerization²¹.

We have purified and characterized an actin filament 'capping protein' from *Acanthamoeba* and suggest that it may play a central role in the regulation of both actin filament polymerization and cross-linking.

Purification of the capping protein

As the capping protein inhibits the low shear viscosity of pure actin filaments, we used a low-shear (<10 s⁻¹) falling-ball viscometer¹⁶ to assay the capping protein during purification. As the first step, crude *Acanthamoeba* extracts were fractionated on DEAE-cellulose as described previously¹⁶. The fractions containing the overlapping peaks of viscosity-inhibiting activity¹⁶, myosin-2 and actin were pooled and actomyosin-2 precipitated²². The resulting supernatant, which contained most of the viscosity-inhibiting activity, was used as the starting material to purify capping protein to near homogeneity. The purification steps included ammonium sulphate precipitation, and gel permeation, hydroxylapatite and ion-exchange chromatography (Fig. 1). The small amount of capping protein which precipitated with the actomyosin-2 could be recovered as a by-product of myosin-2 purification by the method of Pollard *et al.*²².

The purified protein from four different preparations consisted of two major polypeptides with MWs 28,000 and 31,000 and a variable minor polypeptide of MW 60,000. The inhibitory activity is probably associated with the polypeptides of MWs 28,000 and 31,000 because they were present in proportion to inhibitory activity over the final DEAE peak, and because the minor contaminants including the 60,000-MW polypeptide

were also present in neighbouring fractions without inhibitory activity.

As shown in Fig. 1, the specific inhibitory activity of the fractions increased about 50-fold during purification of the two polypeptides by more than 100-fold, suggesting that the protein was partially inactivated during purification. The presence of some gelation-factor activity in the crude fractions (Fig. 1) made calculation of yields somewhat difficult, but we estimated the overall yield to be ~10%. In terms of mass, we obtained ~4 μ g of purified capping protein per g of wet cells.

Physical properties of the capping protein

The two polypeptides constituting the purified protein were present in the ratio of 1.5 (31 K) to 1 (28 K) judging from the intensities of the two bands on Coomassie blue-stained electrophoretic gels. In addition there was ~10% of a 60 K polypeptide. The Stokes' radius of native capping protein was 4.0–4.4 nm measured by the method of Ackers²³ using gel-permeation chromatography in either 20 mM imidazole or 0.6 M KCl. A spherical protein of this size would have a molecular weight of about 120,000 to 160,000. Thus the native protein could consist of up to two copies each of the two polypeptides. The capping protein is soluble in both low and high ionic strength buffers at neutral pH, but its activity is more stable in 50 mM KCl than in 20 mM imidazole alone. The absorption spectrum of the capping protein has a maximum at 277 nm and a minimum at 250 nm. The extinction coefficient at 280 nm is ~0.86 cm¹ ml mg⁻¹.

Binding of capping protein to actin filaments

Capping protein does not pellet by itself, but binding to actin filaments was demonstrated in experiments where 4.8 μ g capping protein (6 μ g total) pelleted at 150,000g for 45 min with 500 μ g of actin filaments in 2 mM MgCl₂. Although binding has not been studied in any detail, this did not represent saturation of capping protein binding sites, because 7.2 μ g capping protein (12 μ g total) pelleted with the same amount of actin.

Effect of capping protein on actin filament polymerization

The polymerization of purified actin monomers consists of a slow nucleation step followed by a rapid elongation step²⁴. We measured the rate and extent of polymerization in an Ostwald capillary viscometer with a high shear rate (>1,000 s⁻¹) which disrupts weak associations between the filaments, making the observed viscosity largely a function of polymer concentration and length distribution. In 20 mM KCl the nucleation step was very slow as shown by the 40-min lag in the time course of the viscosity change for actin monomer alone (Fig. 2b). The elimination of this lag by added actin filaments confirmed that it was due to nucleation (Fig. 2b). The rapid rise in viscosity after nucleation was due to elongation of the filaments. In 2 mM MgCl₂ both of these steps were faster (Fig. 2a).

Purified capping protein had a complex effect on actin polymerization. Paradoxically, it both promoted nucleation and inhibited the rate of growth of actin filaments (Fig. 2a,b). These effects were most apparent in conditions where polymerization was slow, such as 20 mM KCl, where very low concentrations of capping protein eliminated the lag phase (Fig. 2b). This demonstrated stimulation of nucleus formation. In 2 mM MgCl₂ the lag was short, but high concentrations of capping protein clearly increased the initial rate of the viscosity change and altered the shape of the curve from sigmoidal to hyperbolic (Fig. 2a).

Although capping protein promoted nucleus formation, it was, at the same time, a potent inhibitor of the rate of actin filament elongation. This inhibition was seen in mixtures of capping protein with pure actin monomers (Fig. 2a,b), but is best illustrated by experiments in which actin monomer polymerization was nucleated by actin filament fragments. The rate

of the viscosity change in the absence of capping protein was much greater than the rate in the presence of capping protein (Fig. 2b).

By observing the direction and extent of actin filament growth at the ends of decorated nuclei (Fig. 3), we found that capping protein inhibited filament elongation by blocking monomer addition at the barbed end of the filaments (Fig. 3). As established previously⁸ actin filaments normally grow bidirectionally with a strong bias for the barbed end (Fig. 3a). In the presence of low concentrations of capping protein, monomer added to the pointed end, but there was no growth at the barbed end (Fig. 3b). Capping protein also blocked monomer addition to the barbed end of actin filaments in the presence of 5 mM MgCl₂, 75 mM KCl and 0.16 mM ATP. This was shown in monomer addition experiments using demembrated microvillus cores²⁵. The rates of growth at the pointed end with capping protein were 1 molecule per s in 20 mM KCl and 6 μ M actin, and 6 molecules per s in Mg-KCl and 3 μ M actin. These rates were similar to normal growth rates at the pointed end.

The capping protein also inhibited the extent of annealing of actin filaments fragmented by sonication (Fig. 2c). The steady-state viscosity is reduced in a concentration-dependent fashion, but the rate of the approach of the viscosity to the steady state was hardly affected.

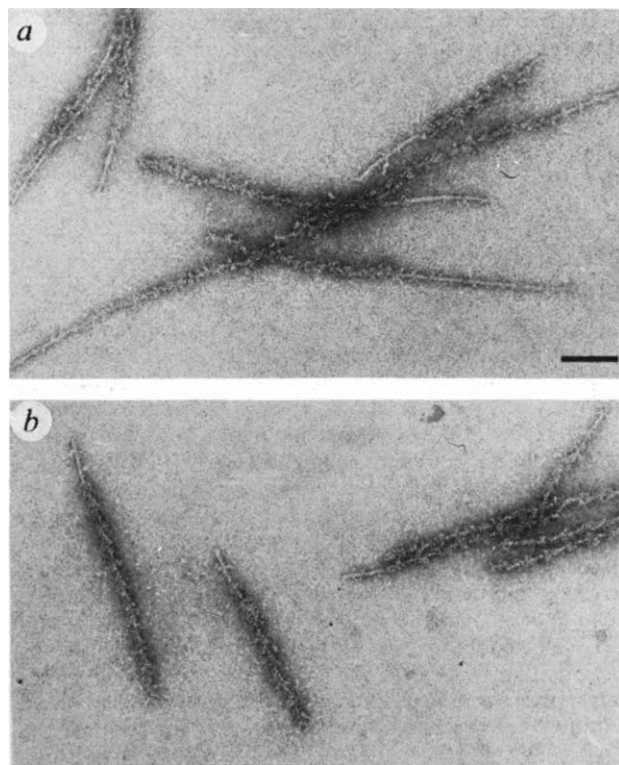


Fig. 3 Effect of filament capping protein on the polarity of actin filament polymerization in 20 mM KCl. The direction of polymerization was determined electron microscopically by adding actin monomers to short actin filaments decorated with an equimolar amount of muscle myosin subfragment-1 which acted as nuclei. These decorated nuclei were reduced in length by passage through a syringe needle. Samples for electron microscopy were negatively stained with 0.75% uranyl formate, pH 4.3. Scale bar, 100 nm. *a*, Control. 50 μ g ml⁻¹ decorated actin nuclei were incubated for 5 min at 22 °C with 100 μ g ml⁻¹ actin monomer freshly prepared by gel filtration in ATP-free buffer¹⁸. The bare filament growth is predominantly from the barbed end of the decorated nuclei. *b*, Effect of capping protein. 50 μ g ml⁻¹ decorated nuclei were incubated with 150 μ g ml⁻¹ ATP-free monomer and 2.2 μ g ml⁻¹ capping protein for 30 min at 22 °C. Note the absence of barbed-end growth and the short bare filaments at the pointed end.

Table 1 Effect of filament capping protein on the steady-state polymer concentration

Capping protein ($\mu\text{g ml}^{-1}$)	Supernate actin monomer ($\mu\text{g ml}^{-1}$)	Calculated polymer ($\mu\text{g ml}^{-1}$)	Specific viscosity (cS)	Reduced viscosity (cS per mg polymer per ml)
0	34	466	0.42	0.90
0.44	59	441	0.38	0.86
1.54	128	372	0.37	0.99
3.08	136	364	0.32	0.88

Steady-state polymerized actin samples from the experiment in Fig. 2a were centrifuged for 20 min at 10^5 r.p.m. in a Beckman Airfuge at 22 °C. Actin filaments pellet in 5 min in these conditions. The fraction of unpolymerized actin in the supernate was determined by quantitative gel electrophoresis. The concentration of polymer was calculated by difference.

The filament capping protein reduced the steady-state high-shear viscosity in a concentration-dependent fashion (Fig. 2). This seemed to be due primarily to a reduction in polymer concentration (Table 1), because the reduced viscosity per mg polymer was not altered significantly by the presence of the capping protein. We also used electron microscopy of negatively stained specimens to measure the lengths of actin filaments formed in the presence or absence of the capping protein. Although the weight-average filament length of samples with capping protein was about 25% less than controls in two different experiments, we were not convinced that the observed difference was meaningful. One problem was that the extent of the reduction in length was not proportional to the reduction in viscosity. Another was that the measured weight-average lengths of identical samples can differ by more than 25% (ref. 18).

Effect of the capping protein on actin filament structure

Visual inspection of negatively stained actin filaments in the absence or presence of enough capping protein to inhibit the low-shear viscosity by more than 95% did not reveal any qualitative structure differences; nor was it possible to detect capping protein at the ends of the filaments. To assess possible structural differences in a more quantitative way, we prepared Mg^{2+} -paracrystals from pure actin filaments (Fig. 4a) and capping protein-actin filaments (Fig. 4c) and analysed electron micrographs of these specimens by optical diffraction (Fig. 4c,d). (Although 50 mM MgCl_2 was used to make actin filament paracrystals, approximately the same amount of capping protein is bound to actin filaments in 2 mM or 50 mM MgCl_2 .) In both cases we selected the 10 best diffraction patterns which showed diffraction spots to at least $(3 \text{ nm})^{-1}$. We measured the layer-line altitudes corresponding to the axial and structural repeats of the actin double helix (see, for an example, ref. 26). The average helix parameters for the two data sets did not differ significantly from each other: the axial repeat was $5.5 \pm 0.1 \text{ nm}$ and the structural repeat was $35.5 \pm 1 \text{ nm}$ in both cases. Furthermore the centre-to-centre spacing of the filaments measured directly on electron micrographs was $6.5 \pm 0.3 \text{ nm}$ for both samples. Qualitatively the intensity distribution along corresponding layer lines was the same. This low-resolution structural analysis suggested that capping protein bound to actin filaments does not alter the helical structure of the filaments substantially.

Effect of the capping protein on actin filament networks

Capping protein inhibited the low-shear viscosity of pure actin filaments (Fig. 5 inset) much more strongly than the high-shear viscosity (Fig. 2a). One microgramme of capping protein reduced the low-shear viscosity of 500 μg actin by 90%. This inhibition of the low-shear viscosity was identical in EGTA and

in 0.1 mM CaCl_2 , so the capping protein was not regulated directly by calcium. Cytochalasin B and capping protein have an additive inhibitory effect on pure actin filament low-shear viscosity; 0.1 μM cytochalasin B reduced the viscosity by about the same amount as 0.5 $\mu\text{g ml}^{-1}$ ($\sim 0.0003 \mu\text{M}$) capping protein.

Capping protein also decreased the low-shear viscosity of mixtures of actin filaments with a new 85,000-MW Ca^{2+} -sensitive gelation factor from *Acanthamoeba*²⁷ (Fig. 5) or with aldolase, a nonspecific actin cross-linker. Low concentrations of capping protein increased the concentration of cross-linker required to form a gel and concentrations of capping protein greater than 1.1 $\mu\text{g ml}^{-1}$ prevented gelation by the *Acanthamoeba* gelation factor entirely (Fig. 5).

Interpretation

We suggest that actin filament capping protein has the following mechanism: it binds to the two actin molecules at the barbed end of actin filaments or it binds to two actin monomers at their barbed poles. In this way it might both promote nucleation, by stabilizing actin dimers, and also inhibit filament elongation by allowing growth in only the slow pointed direction. The formation of networks by pure actin filaments might be affected if the presence of capping protein at the barbed ends of filaments eliminates the cytochalasin-sensitive interactions, postulated¹⁸ to be between the ends and the sides of the filaments. A second but less likely possibility for the effect of capping protein on actin filament networks is that it simply reduces the length of the filaments, as others have suggested for the action of cytochalasin B (ref. 17) and gelsolin¹².

Capping protein is similar to cytochalasin B in that both block actin monomer addition at the barbed end of actin filaments¹⁸ and both strongly inhibit the formation of networks by actin

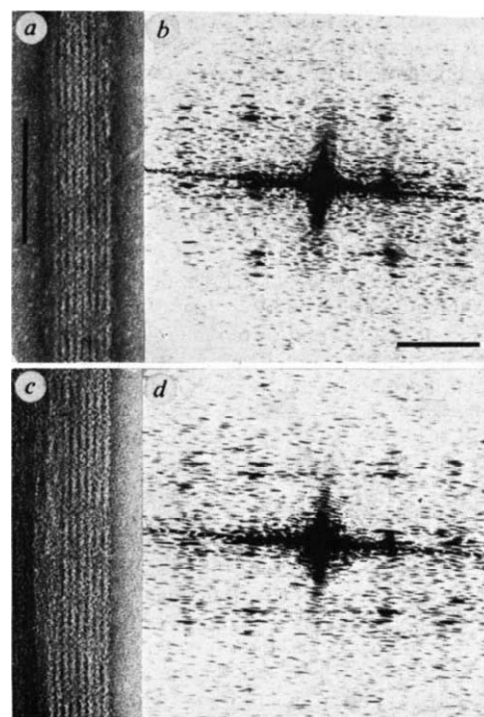


Fig. 4 Electron micrographs (a, c) and optical diffraction patterns (b, d) of actin (a, b) and capping protein-actin (c, d) paracrystals. Gel-filtered actin, 0.5 mg ml^{-1} , was first polymerized for 10 min at 22 °C in 50 mM KCl, 2 mM $\text{K}_2\text{S}_2\text{O}_8$, 10 mM imidazole, pH 7.5, either alone or with 3 $\mu\text{g ml}^{-1}$ of capping protein. MgCl_2 was then added to a concentration of 50 mM for 10 min to induce paracrystal formation. Samples diluted to 50 $\mu\text{g ml}^{-1}$ were applied to glow-discharged carbon-coated grids and stained with 0.75% uranyl formate, pH 4.3. Optical diffraction patterns were recorded from selected areas of electron micrographs of the paracrystal, typically four to six filaments wide and six to 12 structural repeats long. Scale bars, 100 nm (a, c), $(5 \text{ nm})^{-1}$ (b, d).

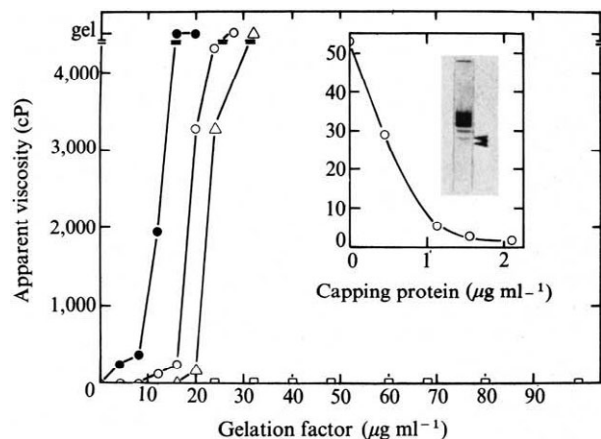


Fig. 5 Effect of filament capping protein on actin gel formation and on the low-shear viscosity of actin filaments. Samples consisting of 0.5 mg ml⁻¹ actin with 0 (●), 0.44 μg ml⁻¹ (○), 0.88 μg ml⁻¹ (△) or 1.1 μg ml⁻¹ (□) capping protein were mixed with various concentrations of an 85,000-MW gelation protein from *Acanthamoeba*²⁷ and polymerized in capillary tubes for 10 min in 2 mM MgCl₂, 1 mM ATP, 1 mM EGTA, 10 mM imidazole, pH 7. The low-shear apparent viscosity was measured with the falling-ball device. Inset: actin alone or with several concentrations of capping protein was polymerized in capillary tubes in the conditions of Fig. 2a legend for 10 min after which the apparent viscosity was measured with the falling-ball device. Incubation for up to 40 min did not change the viscosity of actin plus 2.2 μg ml⁻¹ capping protein. The electrophoretic gel illustrates the composition of a sample with 0.5 mg ml⁻¹ actin with 1.1 μg ml⁻¹ capping protein.

filaments^{17,18}. In contrast, capping protein, but not cytochalasin B, promotes the formation of filament nuclei by actin monomers and inhibits annealing of actin filaments.

Both the *Acanthamoeba* capping protein and muscle β-actinin⁶ inhibit polymerization rates and annealing, but they seem to be different molecules. The subunit molecular weights and Stokes' radii differ, the two proteins may be bound at opposite ends of the filament, and β-actinin is not a potent inhibitor of the low-shear viscosity of pure actin filaments⁶.

Although physiological functions cannot be proven by our purely biochemical experiments, the properties of the capping

protein molecule suggest several functions. For example, capping protein could prevent actin filament self-associations and thereby permit regulation of cytoplasmic consistency solely by variation of the extent of filament cross-linked by gelation factors^{14,16,27}. This could be accomplished without changing the extent of actin filament polymerization or the size of the filaments. Second, the concentration of capping protein could determine the number of actin filaments in the cell. This would occur if filaments with blocked barbed ends are more stable than filaments with free barbed ends, or if spontaneous nucleation is prevented by the binding of profilin to actin monomers³. Finally, and perhaps most interestingly, capping protein could provide a link between the barbed end of actin filaments and various cellular structures to which they are attached, such as membranes.

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LETTERS

B2 1141 + 37: a giant radio galaxy with remarkable radio and optical properties

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The radio galaxy B2 1141 + 37 is remarkable for both its radio and optical properties. The radio source has a double structure for which the ratio of the separation between the components to their dimension is exceptionally large. Moreover, our recent measurement of the redshift gives $z = 0.115$ giving a separation between the components of 1.0 Mpc. This makes 1141 + 37 one of the largest radio structures known. The optical identification which has been proposed is a tight chain of galaxies which are

probably gravitationally bound. If this is the case, the radio galaxy must have some precession and/or rotation motion around the centre of mass of the group with important consequences on the structure and evolution of the radio source. We report here on the redshift measurement and on some VLA observations at 4.9 GHz.

The radio source is formed by the two sources of the Bologna Catalogue B2 1141 + 37 A and B, and is also 4C37.42. It is one of the objects in the sample of radio galaxies which was analysed by Meier *et al.*¹ to obtain the luminosity function of radio galaxies, and it is the only object in that sample for which the redshift was not measured.

The map² of B2 1141 + 37 obtained at 1,415 MHz with the Westerbork Synthesis Radio Telescope shows two intense components of nearly equal intensity separated by 264 arc s and with dimensions of 65 × (<30) arc s for the NE component and 20 × (<30) for the SW component. No third component was detected along the line joining the two bright components. Polarization of 4% and 3% at 1,415 MHz has been detected in the SW and NE components respectively⁷.

The radio source was re-observed at 4,900 MHz with the VLA in August 1978 during observations of the central sources

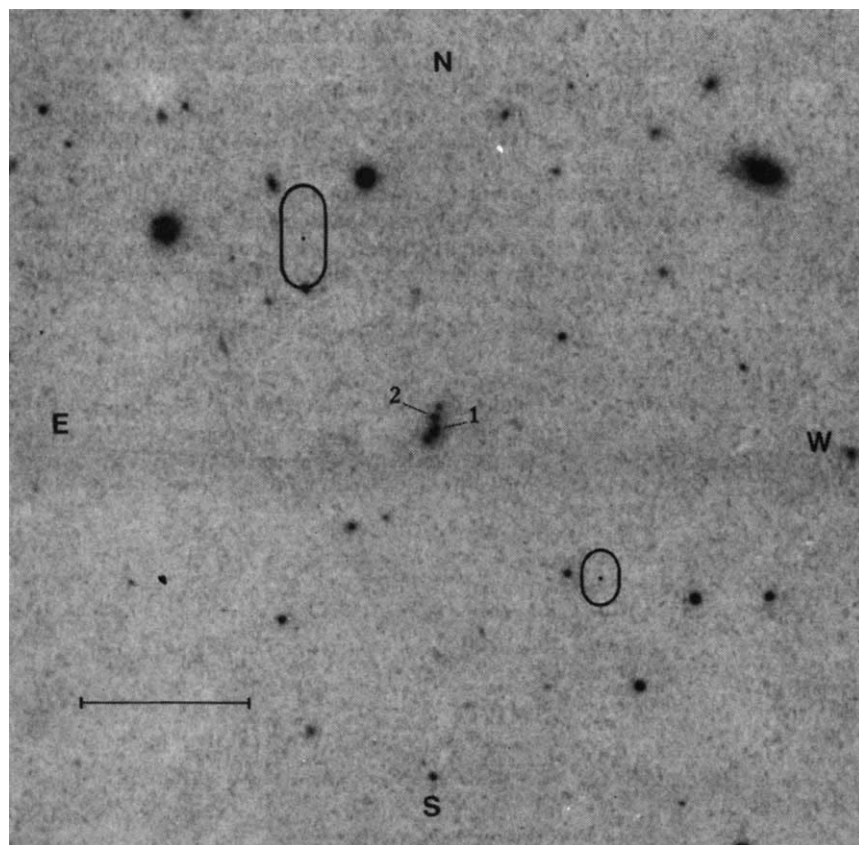


Fig. 1 Radio lobes observed at 1,415 MHz (ref. 2) on a print of the Blue Sky Survey photograph. Scale bar, 100 arc s.

of several radio galaxies from the Bologna Catalogue³. Five integrations of 5 min were done, but after removing the scans affected by phase shifts due to stormy weather, the effective integration time was 17 min. The map obtained with a grid of 3×3 arc s and a gaussian taper falling to 50% at 2.5 km in the (u, v) plane shows two radio lobes with maxima at the positions indicated in Table 1 and exactly coinciding with the maxima in the 1,415 MHz map². The intensities of the NE and SW lobes at

Table 1 Parameters relative to B2 1141+37 A, B

	NE	SW
Coordinates of the peak at 4.9 GHz: (1950)	11 h 41 min 57.2 s 37°26'58"	11 h 41 min 41.6 s 37°23'42"
Radio power of the two components (Jy):		
408 MHz	2.79	2.35
1,415 MHz	0.97	0.84
5,000 MHz	0.23	0.18

Table 2 Comparison of 3C236, Cyg A, and B2 1141+37 A, B

Source	3C236 [†]	Cyg A [‡]	B2 1141+37 A, B [‡]
Distance* (Mpc)	554	320	687
Linear size (Mpc)	5.7	0.16	1.0
Radio power (WHz ⁻¹ sr ⁻¹)	2.6×10^{25}	2.7×10^{27}	1.8×10^{25}
Component properties			
Volume (cm ³)	3.5×10^{72}	3.3×10^{65}	4.7×10^{70}
Minimum energy (erg)	5.4×10^{59}	8.9×10^{57}	1.6×10^{59}
Energy density (erg cm ⁻³)	1.6×10^{-14}	2.7×10^{-8}	3.4×10^{-12}
Equipartition magnetic field (G)	6.3×10^{-7}	8.2×10^{-4}	9.3×10^{-6}

*Assuming $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$.

[†]Ref. 5.

[‡]See text.

4,900 MHz are 0.23 and 0.18 Jy, respectively. These values are both ~ 1.5 times smaller than the values obtained by extrapolating to 4,900 MHz the spectrum defined by the flux density at 408 and 1,415 MHz, $f_\nu \propto \nu^{-0.84}$, of the two radio lobes. This suggests that some flux is missing in the 4,900-MHz map, and this is probably caused by the fact that the shortest baseline spacing of the VLA observations was 480 m. No central source brighter than 0.03 Jy has been detected at the location of the optical identification.

A tight chain of four galaxies located very close to the mid-point between the two radio components has been proposed as the optical identification². We have recently measured the redshifts of the two brightest galaxies in the chain, galaxies 1 and 2 in Fig. 1, with the image intensified disector scanner of the 4 m telescope at Kitt Peak National Observatory. Galaxy 1 shows the *K*, *H*, and *G* features and the [O II] $\lambda 3,727$ emission line at a redshift $z = 0.1145 \pm 0.0005$. The scan of galaxy 2 shows the *K* and *H* lines at $z = 0.1155 \pm 0.005$. Galaxy 1, being the brightest and having a spectrum with at least one emission line, is likely to be the radio galaxy among the four members of the chain. Taking $H = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$, the distance to the radio source is 687 Mpc and 1 arc s corresponds to 3.3 kpc. The distance between the edges of the two radio lobes is, therefore, 1.0 Mpc making it one of the largest radio sources known. The minimum energy in the radio lobes, the energy density, and the equipartition magnetic field have been calculated following the procedure of McDonald *et al.*⁴ and making no allowance for the presence of high energy protons. The calculations are based on the flux density at 604 MHz which is calculated by interpolating the radio spectrum defined by the flux density at 408 and 1,415 MHz, $f_\nu \propto \nu^{-0.84}$. The values corresponding to the NE component are listed in Table 2 together with the values of the same parameters for the E component of 3C236 (ref. 5) and for the outer compact component in the W lobe of Cyg A (component 'D' in ref. 6). For Cyg A we have calculated the energy from the flux measured⁶ at 5 GHz extrapolated to 604 MHz with a power law spectrum $f_\nu \propto \nu^{-1}$ and assuming that this flux comes from a region which has the same dimensions as

those measured⁶ at 5 GHz. Comparison of the values of the energy density in the radio lobes of 1141+37 and in the outer compact component of Cygnus A indicates that 1141+37 is in a more relaxed state.

A full synthesis map of 1141+37 would be particularly interesting for several reasons. First, there are very few radio galaxies with comparable dimensions. Second, the inner radio structure should contain information related to the motion of the parent radio galaxy in its group. The closeness in redshifts of galaxy 1 and 2 and the tight group which they form in the plane of the sky with the other two smaller galaxies suggest that this is a gravitationally bound group. If this is the case, the radio galaxy, probably galaxy 1, has some precession motion and/or rotation motion around the centre of mass of the group. The direction of the beam along which the particles have been ejected must, therefore, have recently undergone some changes influencing the shape and the evolution of the radio structure between the outer lobes and the parent radio galaxy.

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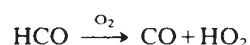
Two-dimensional model calculations of stratospheric HCl and ClO

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Most previous attempts to estimate the effects of atmospheric contaminants on stratospheric ozone have involved one-dimensional models of the troposphere and stratosphere. Estimates of the distribution of HCl and ClO with height have, however, failed to match the measured concentration profiles¹. Hence, there has been much interest in the development of two-dimensional atmospheric models which, by including variations with latitude and seasonal effects, might remove these discrepancies. We have developed such a two-dimensional model which incorporates the refinements of the most advanced one-dimensional models; we now report that, contrary to expectation, the calculated profiles of HCl and ClO are insignificantly different from those of comparable one-dimensional models.

Our model includes 30 active chemical species (H_2O , CH_4 , H_2 , CO , CH_2O , NO , NO_2 , HNO_3 , N_2O , NO_3 , N_2O_5 , HO_2NO_2 , N , HCl , ClO , Cl , ClONO_2 , HOCl , $\text{O}(^3\text{P})$, $\text{O}(^1\text{D})$, O_3 , H_2O_2 , OH , HO_2 , H , CFCl_3 , CF_2Cl_2 , CCl_4 , CH_3Cl , CH_3CCl_3) and all chemical reactions and reaction rates from the current NASA kinetics documentation² applicable to these species, except that a single pair of reactions in the model methane oxidation sequence is assumed to describe the instantaneous fate of CH_3 and HCO radicals:



More recent reaction rates for $\text{OH} + \text{H}_2\text{O}_2$ (refs 3, 4) and HO_2NO_2 photolysis⁵ have, however, been used.

In this model, the transport of all chemical species is calculated separately, except in the odd-oxygen family (O_3 , $\text{O}(^3\text{P})$ and $\text{O}(^1\text{D})$) and H and N atoms (the latter two are assumed to be in photochemical equilibrium). The domain of the model is from pole to pole and from 0 to 55 km. Solar declination angle, temperature field, number density field and transport coefficients are specified for quarter-year intervals to allow for seasonal variations. The treatment of diurnal effects and the approach used for Rayleigh scattering of solar radiation are described by Miller *et al.*⁶. Rainout of soluble species occurs below 10 km with a 5-day time constant.

Mean meridional circulation is parameterized using the advective circulation field of Murgatroyd and Singleton⁷, with a scale factor of 0.4. Dunkerton⁸, Matsuno⁹ and Schoeberl (personal communication) have indicated that, with downward scaling to account for improvements in calculated stratospheric heating rates¹⁰, this field should reasonably approximate the so-called lagrangian mean motion. The lagrangian mean, which resembles a simple Brewer–Dobson^{11,12} cell, combines the zonal eulerian mean motion with a meridional advective contribution arising from planetary waves⁹. This is the first attempt in a complete two-dimensional chemical model to simulate directly the effects of the lagrangian mean circulation.

The eddy diffusion parameterization is basically that of Luther¹³, with the latitudinal coefficient K_y , adjusted below 10 km to give a realistic interhemispheric mixing time of ~14 months (ref. 14). This adjustment produces negligible changes in calculated stratospheric profiles of HCl and ClO. The vertical eddy diffusion coefficient K_z is the most uncertain aspect of Luther's parameterization, and has been replaced by that of Hunten¹⁵ for all seasons throughout the model stratosphere.

Spatial derivatives for transport are approximated by a second-order finite difference representation that automatically conserves mass. Grid resolution is 5 km vertically and 0.2 in the sine of latitude. Fluxes across upper (55 km) and polar boundaries are set to zero for all species. Ground-level species mixing ratios and fluxes are provided as a function of latitude.

The time-dependent integration uses an implicit finite difference formula, second order in the time increment with respect to transport, and first order for chemistry. At each integration step, the finite difference formula is solved by a Newton–Raphson iteration until convergence is achieved. The calculations are performed on a CRAY 1 computer.

Figure 1 compares the calculated latitudinal variations of the N_2O volume mixing ratio with a selection of the available measurements^{16–21}. Agreement is excellent in all latitude bands examined. Results for other upward-moving species such as CFCl_3 , CF_2Cl_2 and CH_4 are similar. The vertical gradient in N_2O mixing ratio is steeper towards the poles than towards the equator, indicating that tropical upwelling associated with the Hadley cell is reproduced qualitatively by the prescribed lagrangian circulation.

Model results for column ozone are consistent with measurements by Wilcox *et al.*²² at all latitudes, as shown by the mid-spring comparison in Fig. 2. Column HNO_3 is considerably improved relative to previous two-dimensional simulations^{23,24}, which typically exceed the measurements of Murcray *et al.*²⁵ by factors of 2–3 at latitudes below 30°. Present calculations fall within the data range in this region, an improvement largely attributable to the use of a lagrangian mean velocity field.

The calculated daytime average vertical profiles for HCl and ClO in May 1977 vary considerably with latitude (Fig. 3). Short-term temporal and local variations might be expected in regions of large spatial gradients. Where the spatial gradient is high for HCl, for example, local variability might be expected below ~30 km, possibly accounting for the observed 'dips' in measured HCl profiles. The seasonal variations suggested by these profiles (for example, 30°N versus 30°S) are, to a first approximation, chemical consequences of changes in the solar

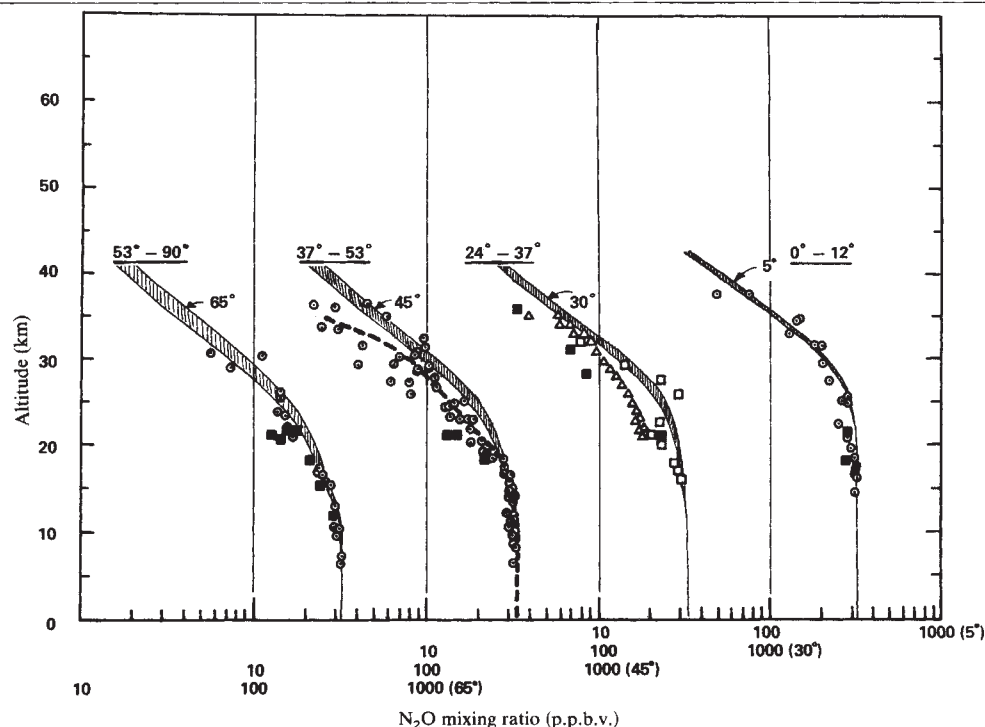


Fig. 1 Calculated vertical profiles of the N_2O volume mixing ratio for several latitudes are compared with a selection of measurements (refs 16–21). $\odot$, Golden *et al.* (1979); $\blacksquare$, NASA Ames Group: Vetter *et al.* (1978), Tyson *et al.* (1978); ---, Fabian *et al.* (1979); $\square$, Ehhalt *et al.* (1975); $\triangle$, Farmer *et al.* (1980); $\blacksquare$, model range over a year.

declination angle rather than of total transport of CIX (= $\text{HCl} + \text{ClO} + \text{ClONO}_2 + \text{Cl} + \text{HOCl}$). Below 20 km, seasonal variability is small because $\text{HCl} \approx \text{CIX}$, but latitudinal variations are quite large.

The range of model-calculated profiles during 1977 for HCl at 30°N latitude (for ~ 1.8 p.p.b. (parts per 10^9) total CIX) is plotted in Fig. 4 together with spectroscopic experimental profiles (from refs 26–30 and Zander, personal communication, 1979), which were obtained between late 1975 and late 1978 at a comparable latitude ($\sim 32^\circ\text{N}$). The *in situ* base-coated filter measurements of Lazrus *et al.*³¹ are believed to include ClONO_2 and HOCl , in addition to HCl , and are therefore not included in Fig. 4. Also, these measurements lie consistently below not only the sum of calculated acidic chloride species, but also virtually all

spectroscopically measured HCl profiles in the region above 28 km.

The calculated HCl profiles in Fig. 4 fall within the envelope of the measurements, exhibiting a qualitatively similar trend of monotonically increasing mixing ratio with increasing altitude. Some measurements also indicate dips that may be due to local variability. The average slope of the calculated profile differs quantitatively from the measurements, with the calculated mixing ratios below 25 km falling at the high end of the measurement envelope, and the values above 25 km deviating towards the low end. These differences are similar to deviations in slope which are observed for HCl in one-dimensional model calculations³².

Figure 5 plots the range of two-dimensional model calculations for daytime average ClO over 1977 at 30°N latitude and the measurements of Anderson *et al.*³³ by *in situ* resonance fluorescence and Menzies³⁴ by laser heterodyne radiometer. If the 28 July 1976 and 14 July 1977 ClO profiles of Anderson are excluded as being unrepresentative of the 'normal' range for that species, then, according to the figure, the model significantly overestimates the abundance of ClO between 25 and 30 km. This overestimation of lower stratospheric ClO is an important problem³⁵, because a major part of the calculated ozone depletion by chlorofluorocarbons occurs below ~ 30 km in the current chemical scheme. Furthermore, it suggests that there may be a problem with CIX partitioning in this altitude region, because ClO represents only a small fraction of total CIX and because calculated HCl is in reasonable agreement with the spectroscopic measurements between 25 and 30 km.

The discrepancy between the calculated and observed ClO profiles in the lower stratosphere is also a feature of one-dimensional modelling calculations. Relative to the one-dimensional case, the present two-dimensional result represents only a minor improvement in agreement between experiment and theory³². Although the pioneering two-dimensional modelling results of Pyle³⁶ have been interpreted as suggesting that the discrepancy would be removed in other two-dimensional simulations¹, this improvement is not found in the present calculation. Other multi-dimensional model results reported by the UK Department of the Environment corroborate this conclusion³⁷. The improved agreement in the calculations of Pyle is related partly to his lower calculated abundance of total CIX in the lower stratosphere and partly to his use of a slower rate for the reaction $\text{HO}_2 + \text{NO}$.

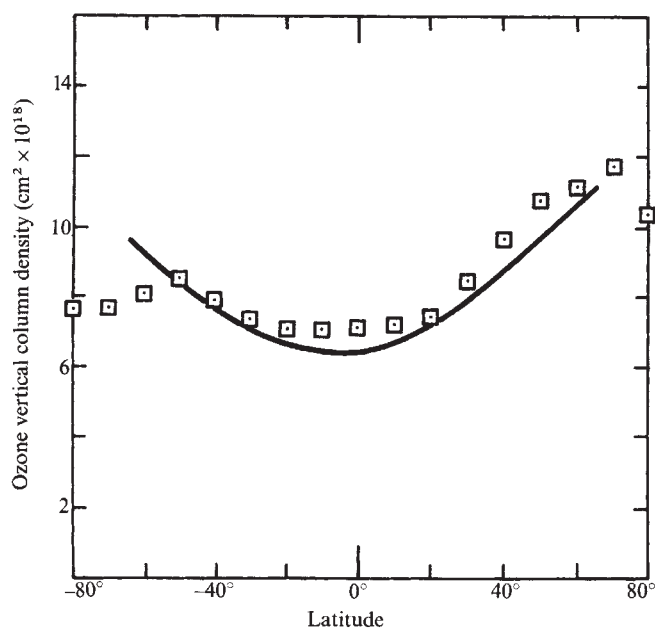


Fig. 2 Model-calculated column ozone as a function of latitude during Northern Hemisphere spring (solid line) is compared with measurements by Wilcox *et al.*²² ($\square$).

Fig. 3 Calculated vertical mixing ratio profiles of HCl and ClO (daytime average) for several latitudes in May 1977.

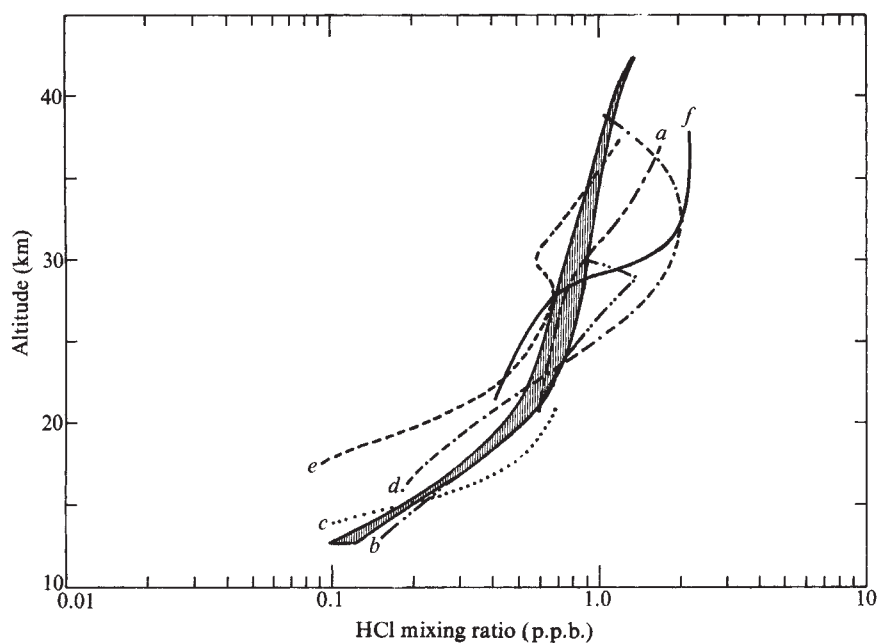
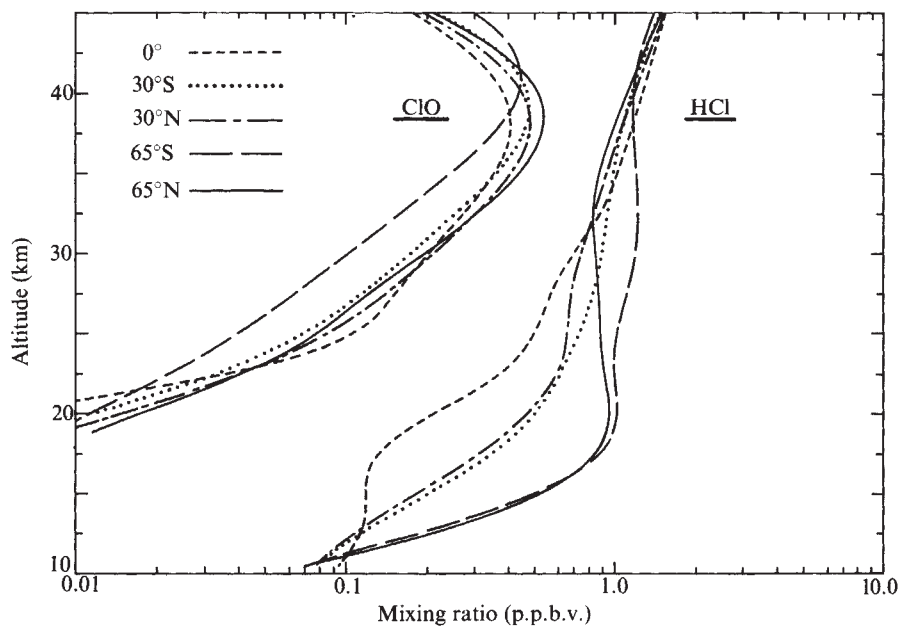
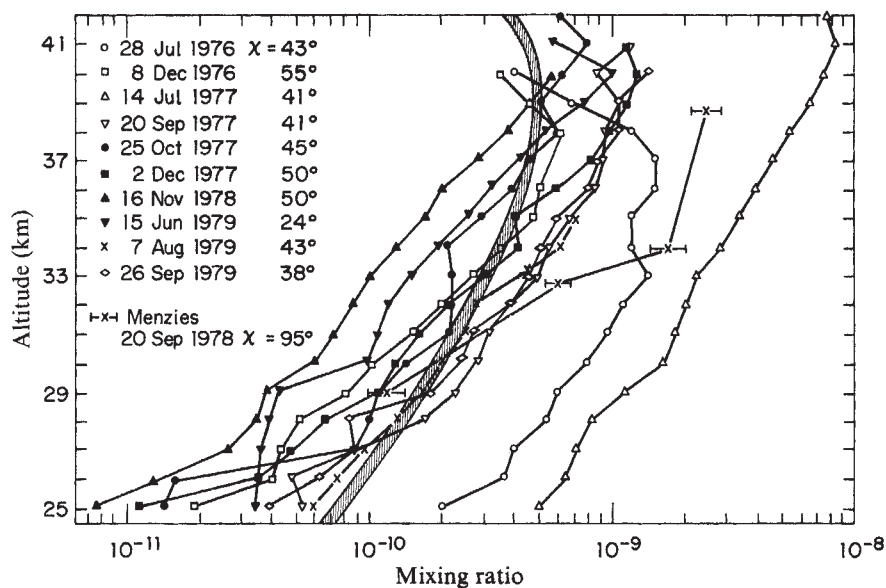


Fig. 4 The range of model-calculated HCl profiles during 1977 at 30°N (■) plotted together with a selection of experimental measurements; a, ref. 30; b, ref. 27; c, ref. 28; d, ref. 29, e, ref. 26; f, Zander, personal communication; made in the same year.

Fig. 5 The range of model-calculated ClO profiles (daytime average) during 1977 at 30°N (■) is plotted together with the *in situ* resonance fluorescence profiles of Anderson *et al.*³³ and the laser heterodyne radiometer measurement of Menzies³⁴.



In the 30–40-km region the agreement between calculated and measured HCl values could be improved considerably by an increase in the maximum ClX mixing ratio from ~1.8 to ~2.5 p.p.b. for 1977. However, such an adjustment would also increase the calculated abundances of both HCl and ClO at altitudes below 30 km, and would therefore produce larger deviations from experiment in the lower stratosphere, given the current chemistry. Furthermore, a mixing ratio of ~2.5 p.p.b. ClX for 1977 would be difficult to reconcile with currently identified source strengths.

Consideration of the behaviour of both HCl and ClO in Figs 4 and 5 suggests that the calculated ratio of ClO/HCl exceeds measurements at all altitudes below 40 km, with the largest deviations occurring in the lower stratosphere. This implies that problems may exist in partitioning of ClX, not merely in the 25–30-km region, but also at altitudes up to 40 km.

The present ClX profiles from a two-dimensional chemical model with advanced treatment of both chemistry and transport indicate that, at 30°N, the comparison with measurements is similar to that obtained with one-dimensional models. In particular, the overestimation of ClO in the lower stratosphere, present in both one- and two-dimensional calculations, is directly related to the large ozone reductions calculated in that region for the fluorocarbon perturbation. As this is also the region where both transport and chemistry are most uncertain, these discrepancies may be reconciled by future revisions to accepted input parameters. At present, discrepancies in total ClX and in partitioning among ClX species imply that the problems are not unique to one-dimensional models and that a reasonable treatment of meridional transport and chemistry does not remove the current disagreements.

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Large rain drops in the bright band

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It has recently been suggested^{1–3}, based on dual-polarized radar measurements, that in the bright-band region, melting snow exhibits the characteristics of rain drops having very large effective drop diameters. It is postulated that snow flakes falling below the 0°C isotherm melt slowly with small water droplets first forming on the outer edges of the flakes; as the snow continues to melt water forms on the surface of the flake, thus it takes on the behaviour of a large rain/ice drop; in the final stages of melting the particles start to collapse into smaller drops. Here another technique for inferring rain-drop sizes⁴ is described which produces results which, being consistent with those above, support the bright-band model.

Based on calculations by Medhurst⁵, rain attenuation can be shown to be a strong function of frequency and drop diameter. In Fig. 1, the ratio of the attenuation at 35 GHz to that at 15 GHz is plotted as a function of drop diameter and it is seen that for small drops, the ratio of the attenuations may be very large but as the drop size increases, the ratio decreases. Although Medhurst's calculations assume hypothetical distributions of rain in that they are for spherical drops all having the same size and temperature, his results are of qualitative value and enable the average drop diameter of the rain to be estimated from measurements of attenuation at two widely separated frequencies. Unfortunately, with the radiometric technique for measuring attenuation, it is not possible to obtain attenuation as a function of distance along the path directly, as

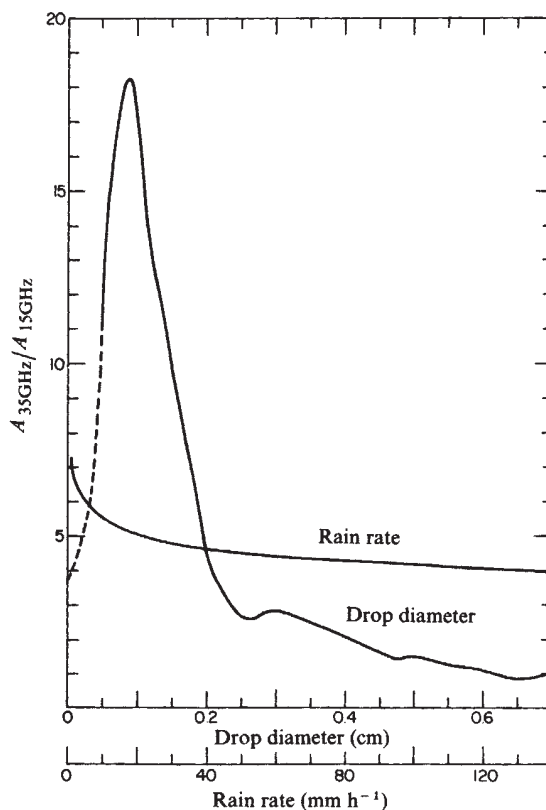


Fig. 1 Ratios of 35-GHz to 15-GHz attenuations as a function of rain-drop diameter and rain rate.

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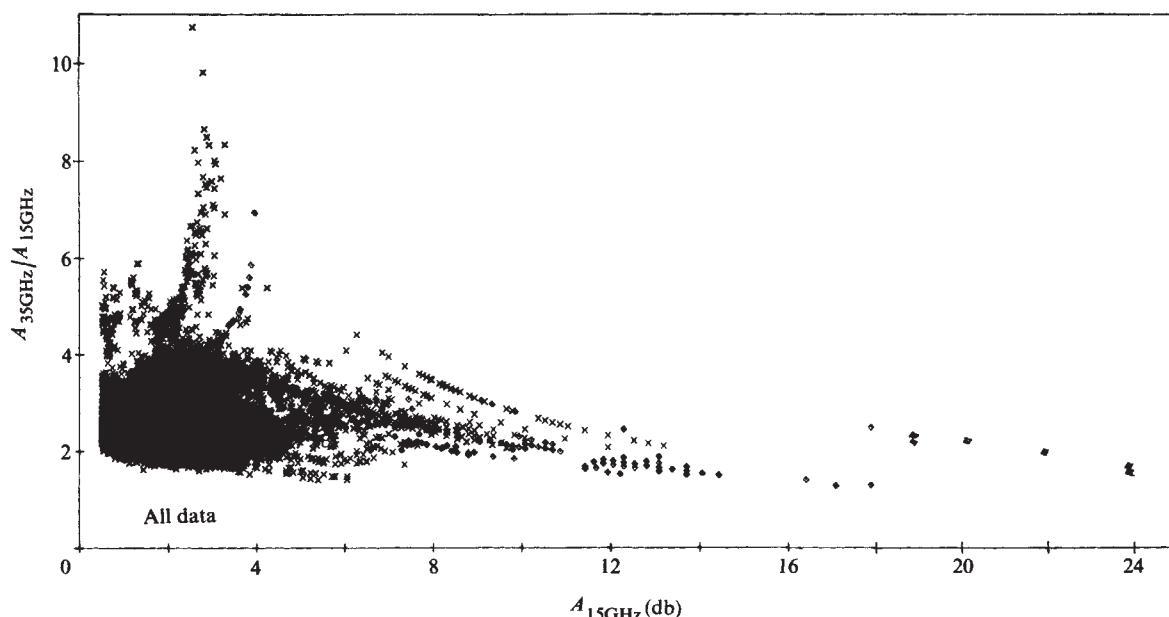


Fig. 2 Ratios of measured 35-GHz to 15-GHz attenuations as a function of 15-GHz attenuation.

can be done with radar. Drop size distributions of rain have been measured at ground level⁶ and a strong correlation between rain rate and the average drop size has been shown; light rain typically consists of small drops of 1- or 2-mm diameter, whereas heavy rain has larger-diameter drops of 3–5 mm. The attenuations corresponding to these drop size distributions can be calculated so the attenuation ratio can also be expressed as a function of rain rate as shown in Fig. 1. Note that the attenuation ratios of ground-level rain would be expected to fall in the range 4–7.

Slant-path attenuation measurements at frequencies of 15 and 35 GHz were conducted in the Boston area, during eight rainstorms with various rain-fall intensities, using the Sun as a source⁴. A dual-frequency radiometer mounted in an 8.8-m paraboloidal antenna was the detector. Antenna beamwidths were approximately 9 and 4 arc min respectively and the centre of the Sun was tracked with an accuracy of at least 0.5 arc min. Over 10,000 simultaneous measurements of attenuations at 15 and 35 GHz were recorded during a year. The ratios of these attenuations are plotted against the 15-GHz attenuations in Fig. 2. Data points corresponding to 15-GHz attenuations below 0.5 dB were not included because they were probably too low to be due to rain and also the measurement accuracy at these low attenuations is poor. If the rain-drop size distributions along the slant path were similar to those which have been measured on the ground, then one would expect the attenuation ratios to be of the order of 6 or 7 for low rain rates (low 15-GHz attenuations) and decrease to about 4 or 5 for the higher rain rates which would correspond to higher 15-GHz attenuations. Of the points which are plotted, the bulk of these have ratios between 2 and 4 and occur for low 15-GHz attenuations. Therefore, these results are not consistent with ground-level rain-drop size distributions but suggest instead that somewhere along the slant path there is a significant contribution to the attenuation which results from precipitation, with characteristics of rain drops having very large effective drop diameters, as noted in the drop-diameter curve of Fig. 1. Because most of the rain along the path occurs below the bright band, the attenuation contribution from the bright band would be expected to be relatively small. Yet, the fact that the attenuation ratios are so low means that there must be very large drops in the bright-band region and therefore drop diameters of the order of 10–20 mm, as suggested by Humphries and Barge², are certainly possible.

Note that it may be advantageous to conduct both dual-frequency attenuation and dual-polarized reflectivity measurements to differentiate between rain and ice. The

attenuation measurement easily distinguishes rain from ice particles as losses due to ice are much smaller; this is not always the case with radar because both ice and rain have high reflectivity. In addition, if the attenuation through the absorbing medium is known, the radar can be more accurately calibrated.

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Proton mobility in ice

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Ice is frequently taken as a model when factors controlling proton transport in hydrogen-bonded molecular networks are discussed. Such discussions have increased with the acknowledgement that proton transfer across cell membranes may play a significant part in energy conversion and storage in biological systems^{1–4} and that this transfer may involve hydrogen-bonded chains spanning the membrane^{5,6}. However, there is still much uncertainty about the basic mode of proton displacement and the external factors which effect bulk proton transport in ice itself. We present here results of a microwave conductivity pulse radiolysis technique which has been used to determine the mobility of bare protons in ice, and thus reduce some of the uncertainty over proton mobility.

The displacement of protons through a Grotthuss-type mechanism can only occur if a subsidiary mechanism is available to wipe out the polarization field produced by dipole reversals which accompany proton jumps. This led Bjerrum to introduce *L* and *D* orientation defects. For a sufficiently high concentration of Bjerrum defects, resistance to proton motion due to bulk polarization should become negligible. In these conditions, therefore, the net drift mobility of protons in an applied field, $\mu(H^+)_{\infty}$, should be equal to the microscopic or virtual mobility,

$M(H^+)$. The latter parameter is of considerable interest because it reflects the basic mode of proton displacement.

However, the situation may be complicated^{7,8} by the formation of immobile complexes between protons and the net negatively charged L defects.



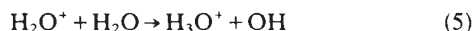
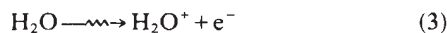
The net drift mobility, even for high concentrations of orientational defects, will, therefore, give only a lower limit to $M(H^+)$:

$$\mu(H^+)_{\infty} = M(H^+) \frac{[H_3O^+]}{[H_3O^+] + [H_3O^+L]} \quad (2)$$

The presently accepted values of the proton mobility in ice at close to the melting point are considerably lower than the estimate of $7.5 \times 10^{-2} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ by Eigen *et al.*⁹ and tend to lie within the range $(0.5-5) \times 10^{-3} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ (ref. 11). The temperature dependence of the mobility is not accurately known. There are indications of increasing mobility with decreasing temperature^{12,13,15}. However, zero activation energy for the mobility is frequently assumed even in recent theoretical models of the dielectric properties of ice¹⁷.

We have studied the change in conductivity which results from pulsed ionization of ice using 3-MeV electrons from a Van de Graaff accelerator. The present results were obtained in the temperature range -5 to -30°C using 50-ns long pulses with a total dose per pulse of $\sim 1 \text{ krad}$ ($\sim 5 \times 10^{16} \text{ eV cm}^{-3}$). The concentration of ions formed was $\leq 1 \times 10^{-6} \text{ M}$. The experimental set-up and background to the microwave conductivity technique used have been described elsewhere^{18,19}. Figure 1 shows the conductivity transient observed in H_2O ice at -7°C .

The results can be discussed in terms of the main ionic processes occurring on irradiation:



The highly mobile ($\sim 10 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$) conduction electrons, e^- , have a mean lifetime towards localization at pre-existing lattice-defect sites, T , of 100 ps at -5°C (ref. 18). This process is responsible for the initial, very rapid decay of the conductivity on termination of the pulse. Further relaxation to give the deeply trapped ($\lambda_{\text{max}} = 680 \text{ nm}$) (refs 20-22), 'solvated' state of the electron, e_{sol}^- , occurs with a mean time of 400 ps (C. D. Jonah and our unpublished results). The relatively slowly decaying

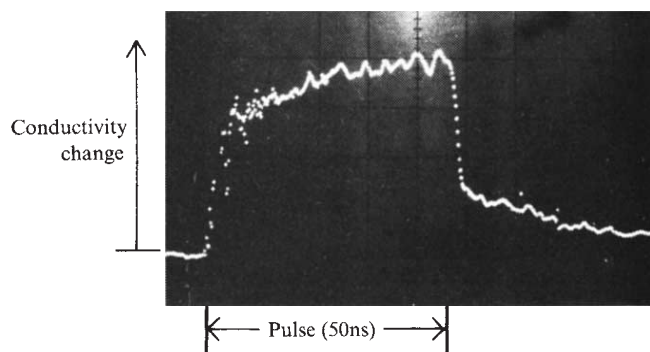


Fig. 1 Transient digitizer trace of the change in conductivity of H_2O ice at -7°C which results on pulsed ionization using a 50-ns pulse of 3-MeV electrons (beam charge 60 nC). The maximum signal height corresponds to a conductivity of $\sim 2 \times 10^{-4} \Omega^{-1} \text{ m}^{-1}$. The signal during the pulse is due mainly to the steady-state concentration of highly mobile conduction electrons which undergo trapping in $\sim 0.1 \text{ ns}$ at this temperature. This electron component is seen to decay very rapidly on termination of the pulse but an appreciable, relatively slowly decaying conductivity transient remains. This transient is attributed to proton conduction.

conductivity transient apparent in Fig. 1 cannot therefore be attributed to the migration of weakly localized electrons through the conduction band or by trap-to-trap hopping.

Because the formation of H_3O^+ through the proton-transfer reaction (5) is expected to take place within a few O-H vibrations²³ or H_2O^+ displacements²⁴ and should certainly be complete well within the nanosecond time scale of the present experiments, we conclude that the slowly decaying conductivity is due to mobile protons.

The present measurements were carried out using microwaves, so that effects on the proton mobility due to polarization of the medium can be neglected. The initial after-pulse conductivity should then be proportional to the product of the proton yield and the virtual mobility $M(H^+)$. Using the known dosimetry, the parameter $G(H^+)M(H^+)$, where $G(H^+)$ is the yield in ions per 100 eV absorbed, can be determined from the conductivity signal after correction for a certain amount of decay during the pulse. At -5°C , the yield times mobility product is determined to be 6.4×10^{-3} and $2.4 \times 10^{-3} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1} (100 \text{ eV})^{-1}$ for H_2O and D_2O ice respectively.

From optical absorption pulse radiolysis studies^{20,22}, the yield of solvated electrons is found to be $1.0 \pm 0.2 (100 \text{ eV})^{-1}$ for H_2O and D_2O ice at -5°C . Taking the yield of protons to be equal to the yield of solvated electrons results in estimates of virtual mobilities of H_3O^+ and D_3O^+ of 6.4×10^{-3} and $2.4 \times 10^{-3} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. As expected on the basis of the initial discussion, the value of $M(H^+)$ lies above the range of drift-mobility values determined from low-frequency dielectric measurements on ice at equilibrium.

Of considerable importance to theoretical models of proton transport is the temperature dependence of $M(H^+)$. For both H_2O and D_2O , the yield times mobility product is found to remain almost unchanged between -5 and -30°C with a slight, $\sim 10\%$, increase being indicated for H_2O . Over this same temperature range, the yield of ions formed, as measured by the solvated electron absorption, decreases considerably^{20-22,25}. A pronounced increase in the virtual mobility with decreasing temperature is therefore indicated.

The electron yield at -30°C is quite sensitive to the conditions of pulse width and total dose used^{20,26}. It can be concluded, however, that the ion yield is at least a factor of 2 lower at the lower temperature. It may then be concluded that the virtual mobility has a negative activation energy of at least 0.15 eV which corresponds to an inverse sixth-power dependence on temperature in the present range.

Such a large negative energy of activation is incompatible with simple theoretical models involving hopping or tunnelling of protons between isoenergetic states separated by a potential energy barrier. A more complex approach, in which interaction between the charged particle as a whole and lattice vibrations is taken into account, has been shown by Gosar and Pintar²⁷ to predict a mobility which sharply decreases with increasing temperature. Fischer and Hofacker²⁸ pointed out that proton conduction can be treated in an analogous way to that used for narrow-band polaron transport. In the low-temperature regime, the narrow-band polaron mobility is given by²⁹

$$\mu = \frac{ea^2}{kT} \pi^{1/2} \nu_0 F(T)^{1/2} \exp[-F(T)] \quad (6)$$

with

$$F(T) = \frac{2W_p}{h\nu_0} \text{cosech} \frac{h\nu_0}{2kT} \quad (7)$$

In equations (6) and (7), a is the intermolecular distance ($2.76 \text{ \AA}$ in the present case), ν_0 is the unperturbed natural frequency of the vibrational mode interacting with the charge, and W_p , the polaron binding energy, is the potential energy gained by the charge due to lattice distortion.

In the case of ice, strong interactions of H_3O^+ with both the oxygen-oxygen lattice mode, $h\nu_0 \approx 0.025 \text{ eV}$, and the restricted rotation or libration mode, $h\nu_0 \approx 0.1 \text{ eV}$, have been suggested²⁸.

The measured value of $M(H^+)$ of $6.4 \times 10^{-3} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ at -5°C is obtained using equation (6) with $W_p/h\nu_0 = 1.72$ or 16.6 for $h\nu_0 = 0.025$ or 0.1 eV respectively. Using the same combinations of parameters, an increase in mobility by factors of 1.9 and 4.6 respectively would be predicted on decreasing the temperature to -30°C . The predicted effect of temperature is of the same magnitude as found. However, because of the large uncertainty in the value of the ion yield at -30°C , the temperature dependence alone cannot be used to specify which lattice interaction predominates. The isotope effect of a factor of 2.7 in the mobility, however, favours the lower energy mode, if the polaron binding energy is assumed to be the same for both isotopes. Thus the decrease in frequency of $\sim 5\%$ for the oxygen-oxygen vibration corresponds to a decrease in mobility using equation (6) by a factor of 1.9. For the librational mode the shift in frequency is $\sim 30\%$ (ref. 30), which would result in the prediction of a mobility a factor of almost 10^4 lower for the heavier isotope.

An interesting aspect of the general narrow-band polaron theory is the prediction of a sharp changeover to a thermally activated hopping motion at higher temperatures^{29,31,32} with, however, an activation energy which decreases with increasing temperature. At sufficiently elevated temperatures, a maximum in the mobility is predicted²⁹. This behaviour is descriptive of the temperature dependence of the excess mobility of protons in liquid H_2O (refs 33, 34).

The proton conductivity signal is found to decay over a period of a few hundred nanoseconds to a much lower level (10–20% of the initial signal) which then further decreases over many microseconds. The slow decay occurs on the same time scale as observed for the solvated electron disappearance in optical studies²² for similar dose conditions and is therefore ascribed to the recombination reaction



The initial relatively rapid decay is attributed to a pseudo first-order reaction of bare protons with L defects, present at a concentration of $\sim 10^{-5} \text{ M}$ (ref. 35) resulting eventually in a considerably lower equilibrium concentration of H_3O^+ .

The ratio between the initial conductivity and the low-level, slowly decaying component is equal to the concentration ratio $([\text{H}_3\text{O}^+] + [\text{H}_3\text{O}^+L])/[\text{H}_3\text{O}^+]$ at equilibrium. The ratios found at -5°C are 8 and 10 for H_2O and D_2O respectively. Combining these ratios with the above values of the virtual mobilities gives the effective drift mobilities $\mu(H^+)_{\infty} = 8 \times 10^{-4}$ and $\mu(D^+)_{\infty} = 2 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. The value of $\mu(H^+)_{\infty}$ lies close to the lower limit of the range of mobility values derived from low-frequency dielectric measurements. Drift mobility estimates larger than $\mu(H^+)_{\infty}$ could be due to perturbation of equilibrium (1) by excessive applied fields.

In summary, using a nanosecond pulse radiolysis microwave conductivity technique, the virtual mobility of protons in H_2O ice has been determined to be $6.4 \times 10^{-3} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, and of deuterons in D_2O ice to be $2.4 \times 10^{-3} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ at -5°C . These values increase by a factor of at least 2 on decreasing the temperature to -30°C . This corresponds to a negative activation energy of at least 0.15 eV. The narrow-band polaron theory is capable of describing the data with the main charge interaction apparently involving the 200 cm^{-1} oxygen-oxygen lattice vibration. Temporary complexing of protons with L defects reaches equilibrium within a few hundred nanoseconds and results in effective drift mobilities of 8×10^{-4} and $2 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ for H_3O^+ in H_2O and D_3O^+ in D_2O respectively at -5°C .

Kinetic and thermodynamic parameters will be discussed elsewhere.

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Evidence against a crystal instability in melting

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Since Born's original suggestion¹, melting has frequently been attributed to a crystal instability although its one-phase approach has been objected to. Using a molecular dynamics computation of the behaviour of a 336-atom Lennard-Jones system, and exploiting the inherent limitations on model size and simulation time, we have found a crystal-liquid instability. It falls outside the density interval for thermodynamic melting, however, and we conclude that it is not true melting. We have also discovered an isothermal liquid-glass transition on compressing the liquid, and this occurs at a density within the transition interval. The results reported here are consistent with the first-order nature of melting and provide new insight into the difference between the crystalline and liquid states.

A sufficiently large system, given enough time, would melt by traversing the two-phase region, whereas a limited model like ours can explore nonequilibrium states. A similar model has, for example, probed the van der Waals loop of the liquid-gas transition (S. Toxvaerd, personal communication). Our three-dimensional system, with periodic boundaries, has been investigated at various densities in the melting region. The studies extended those of Damgaard Kristensen², using the same model and the same reduced units. (The diatomic Lennard-Jones interaction energy is $V_{\text{LJ}} = \epsilon((r_0/r)^{12} - 2(r_0/r)^6)$; reduced units are used throughout—lengths in units of r_0 , energies ϵ , temperatures ϵ/k_B , densities r_0^{-3} , pressures ϵ/r_0^3 .) An equation of state was first established for the face-centred cubic (f.c.c.) phase for densities 1.20–1.35 and temperatures 0.5–1.0. All state points were derived on the basis of 5,000 computational time steps, each of duration 0.003 in reduced units ($2 \times 10^{-14} \text{ s}$). The zero-pressure and higher-pressure isobars all displayed divergence of the isobaric volume expansion coefficient for the (metastable) f.c.c. phase, as implied by the vertical tangent of each isobar, and this defined an unequivocal

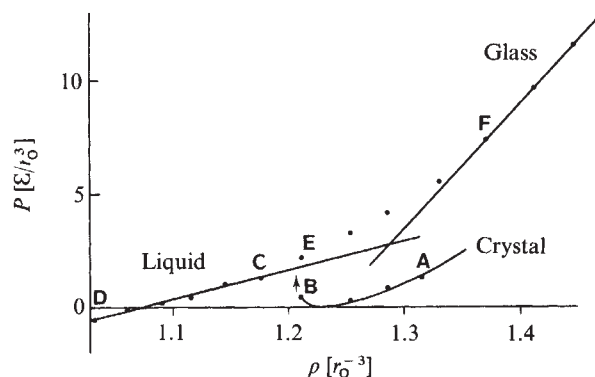


Fig. 1 The $T = 0.77$ isotherms of crystal, liquid and glass with the experimental points indicated. The arrow indicates the position of the crystal instability, and the intersection of the extrapolated lines locates a formal glass transition density. A–F, The expansion and compression sequence of states described in Fig. 2.

thermodynamic stability limit³; however, for the small system investigated the limit can be bypassed slightly⁴. A typical isotherm for $T = 0.77$, starting with the f.c.c. state is shown in Fig. 1. A discontinuous jump in pressure is observed at a density of 1.22. (All isotherms exhibited discontinuities which were sharp within computational limits, the discontinuity density increasing with temperature.) Beyond the discontinuity, pressure again decreased with density. The isotherm was retraced if the density was then increased, but the pressure rose continuously and passed the discontinuity with no detectable change of slope. A change in slope was encountered, however, at a higher density, as shown in Fig. 1.

The pair distribution function, $g(r)$, and a bond-angle distribution function, $f(\theta)$, defined elsewhere⁵, were monitored for all the states indicated in Fig. 1. These functions are shown in Fig. 2. For $g(r)$, the $\sqrt{2}$ -peak, a signature of the f.c.c. structure, a remnant flat portion at B, was totally absent at C. States C to F had liquid-like $g(r)$, with the suggestion of second-peak splitting present at F. The well defined second-peak splitting reported for hard sphere model glasses⁶ was smeared out, presumably by

thermal vibrations. We conclude that the discontinuity is associated with a crystal–liquid transition and that a liquid–glass transition is obtained by compressing the liquid. The glass transition density increased slightly with increasing pressure and temperature relative to the zero-pressure transition density located by Damgaard Kristensen². For $f(\theta)$, there were more pronounced differences between B and C: the 90° peak and the 150° depression, still both seen in B, were absent in C. Because the 90° bond angle is a characteristic of the f.c.c. structure, this supports our conclusion that the discontinuity corresponds to crystal breakdown. Here too, E and F differed markedly: the 60° and 110 – 120° peaks were much sharper in F, and the shoulder at 150° more pronounced, both features are glassy-state characteristics⁵.

Using a Monte-Carlo technique to locate free energy equality, and hence melting pressures for given melting temperatures, Hansen and Verlet^{7,8} derived the fluid and solid densities at melting. Because their model presumably had similar limitations, such as the suppression of long-wavelength fluctuations, imposed by the periodic boundaries, comparison of their results with our own seems reasonable. Clearly, however, both approaches should be applied to larger models when this becomes computationally feasible. For the melting temperature 0.75, which is within computational limits of our $T = 0.77$, they found $\rho_{\text{cryst}} = 1.38$ and $\rho_{\text{liq}} = 1.24$ in our units. Our instability lies at $\rho_{\text{inst}} = 1.22$ for $T = 0.77$. Moreover, their ρ_{cryst} and ρ_{liq} values rise with temperature much faster than does our ρ_{inst} , so the latter would become progressively further removed from Hansen and Verlet's melting interval. This indicates that the observed instability density is not connected with the density at which the critical liquid nucleus becomes comparable in size to the system; if that were so, $\rho_{\text{cryst}} - \rho_{\text{inst}}$ would be expected to be approximately constant. Furthermore, nucleation, albeit of crystal, has been observed⁹ in a system of size comparable with ours. We suggest therefore that there is no essential connection between crystal instability and thermodynamic melting. The observed hysteresis, however, is consistent with approaches to melting which assume no intrinsic correlation between the properties of the crystalline and liquid phases, as discussed recently by Ziman¹⁰.

Two important variants of the crystal instability approach have involved free volume¹¹ and dislocations¹². The present study allows the relative merits of these models to be evaluated.

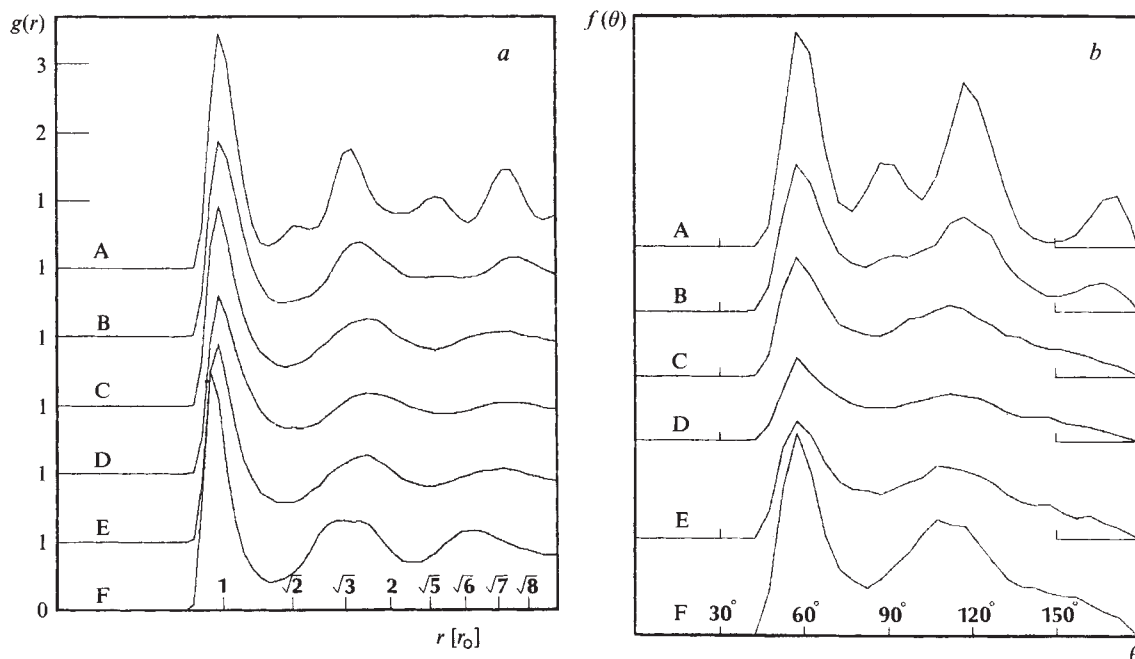


Fig. 2 *a*, Pair correlation functions, $g(r)$, for the six states indicated in Fig. 1. *b*, Bond-angle distribution functions, $f(\theta)$, for the six states indicated in Fig. 1.

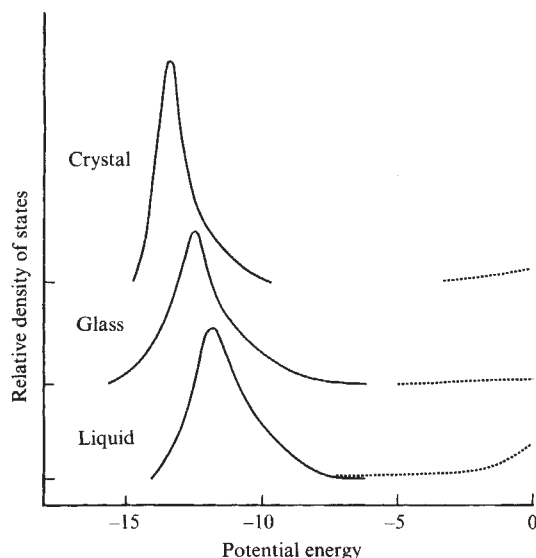


Fig. 3 Potential energy density of states spectra for the normal (solid curve) and hole (dotted curve) states. The three situations (shifted vertically with respect to one another, for clarity) correspond to states A (crystal), F (glass) and E (liquid) indicated in Fig. 1, and the curves extend over the ranges for which states were detectable. For the liquid, the maximum of the normal spectrum lies at -5.97 , while the minimum of the hole spectrum occurs at -7.09 . The curves all have the same (arbitrary) ordinate scale.

The existence of a virtual glass point, located in the thermodynamic density interval for melting, suggests that this transition occurs when the free-energy loss in disordering is just offset by the free-energy gain due to increased atomic mobility. Survival of the crystal at a density lower than ρ_{liq} shows that disorder is a prerequisite for such higher mobility, while the isothermal liquid-glass transition reveals the connection between mobility and density in the disordered state. Stimulated by the recent local free-volume approach of Cohen and Grest¹³, we have computed two potential energy spectra: one for actual energies

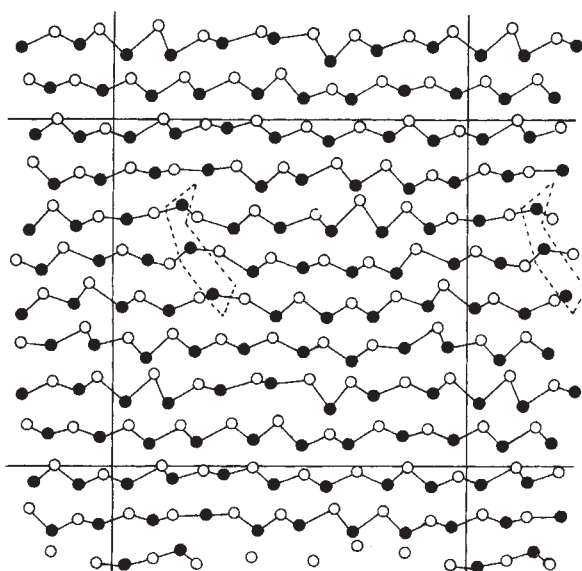


Fig. 4 Atomic positions in two adjacent close-packed layers, corresponding to state B of Fig. 1. Linkage reversal, in which the angle subtended at an atom by its immediate neighbours passes through 180° (changing the zig-zag pattern to zag-zig), permits detection of (Shockley partial) dislocations¹². The dotted line indicates the approximate position of a dislocation loop found in this manner. The long straight lines indicate the periodic boundaries.

of the atoms during the simulation, and one for the energies that atoms would have if moved to the centres of the closest and largest regions of local free volume. These 'normal' and 'hole' spectra for states A (crystal), E (liquid) and F (glass) are shown in Fig. 3. Only for the liquid do the two spectra overlap. In this case, atoms seem to have sufficient energy to take advantage of the choice of position provided by local free volume, and the distinction between normal and hole states is no longer well defined. This is presumably the origin of the enhanced atomic mobility. Finally, using an earlier dislocation-detection procedure¹², we find that the observed crystal-liquid instability is dislocation mediated. No dislocations were observed at densities above state B, but these defects appeared (and rapidly proliferated) on reaching B, as shown in Fig. 4. This result is in conflict with the dislocation theory of melting.

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Low-temperature preparation of refractory alloys

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The escalating cost of energy has stimulated interest in the preparation of refractory and ceramic materials by low-temperature methods. Such methods are particularly attractive for the preparation of catalysts because the products are more finely divided than those obtained from conventional solid-state reactions, and they are also of academic interest because they provide the opportunity to examine some previously inaccessible low-temperature areas of phase diagrams. So far, interest has focused mainly on the synthesis of mixed metal oxides by the ignition of precursors which have been precipitated from aqueous solution¹ or freeze dried², although platinum metal alloys have also been prepared, for example by the reduction of salts which have been impregnated on a silica support³. I report here the low-temperature synthesis of the refractory alloy Mo-W by precipitation from aqueous solution followed by hydrogen reduction.

The melting points of Mo and W are $2,610^\circ\text{C}$ and $3,410^\circ\text{C}$ respectively. They form a continuous body-centred cubic solid solution in which the lattice parameter varies linearly with composition⁴ between $3.1472\text{ \AA}$ (Mo) and $3.1648\text{ \AA}$ (W). The alloys are typically prepared from the melt or by sintering the metals in a powder form at $\sim 2,300^\circ\text{C}$.

In the present experiments, ammonium paramolybdate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$, and ammonium paratungstate, $(\text{NH}_4)_{10}\text{W}_{12}\text{O}_{41}\cdot 5\text{H}_2\text{O}$, were dissolved in hot water and then precipitated by adding the cooled solution to a large excess of ethanol. Examination of individual particles of the precipitates by X-ray emission analysis in a Jeol 100CX TEMSCAN analytical electron microscope revealed a surprising degree of homogeneity in the Mo-W distribution at this stage. The alloys

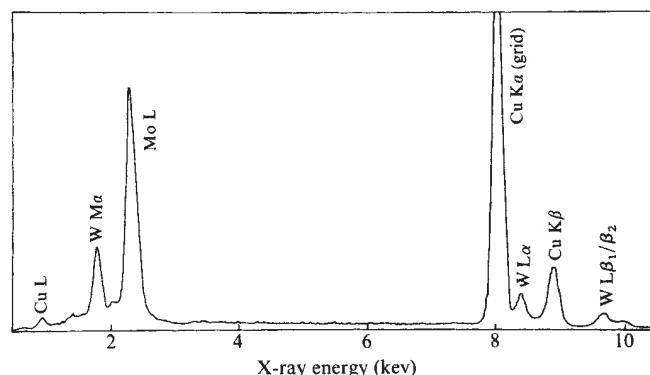


Fig. 1 X-ray emission spectrum from a thin crystal of $\text{Mo}_{0.6}\text{W}_{0.4}$ supported on a copper grid. Operating voltage, 100 keV.

were obtained by reducing the precipitates in hydrogen for ~5 h at 700 °C; reduction can even be effected at 500 °C but the product is pyrophoric. The thermal decomposition of the precipitates in air at 500 °C led to the formation of mixed metal oxides in the system $\text{MoO}_3\text{--WO}_3$. The preparation of these oxides has previously been carried out by a solid-state reaction between MoO_3 and WO_3 at temperatures up to 1,100 °C for 3 days⁵.

The chemical composition of the alloy may be controlled by changing the composition of the starting solution and samples were prepared at the following compositions: 13, 37, 60, 79, 88 and 94 atom % Mo. Individual alloy particles were examined in the analytical electron microscope to monitor the composition and homogeneity, and Fig. 1 shows a typical X-ray emission spectrum. In the thin-crystal limit, the W:Mo ratio is directly proportional to the ratio of appropriate X-ray emission intensities⁶, for example $\text{WM}\alpha : \text{MoL}$, and the results obtained from an examination of several thin crystals at each composition are presented in Fig. 2. The error bars represent the range of $\text{WM}\alpha : \text{MoL}$ ratios observed at each composition. Two of the alloys, at 37 and 79% Mo, are noticeably less homogeneous ($\pm 4\text{--}5\%$ Mo) and this is reflected in their X-ray powder patterns which exhibit broader lines. However, the results clearly

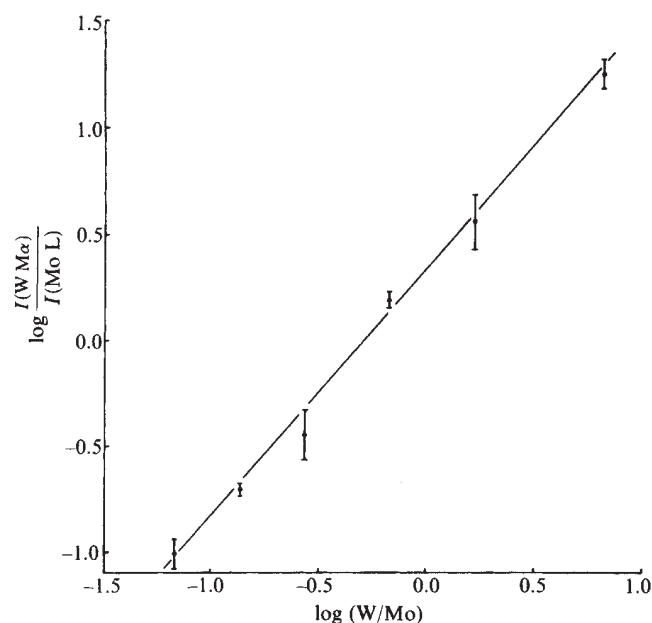


Fig. 2 The measured $\text{WM}\alpha : \text{MoL}$ intensity ratio for the alloys versus the W:Mo ratio in the starting solution. Use of a log-log plot distributes the points more evenly.

indicate the preparation of alloys across the complete composition range and are in accord with the thin-crystal approximation. The electron optical study also revealed a small particle size in the range 0.1–10 μm .

The alloys containing 13, 60, 88 and 95% Mo gave sharp Guinier X-ray patterns with bcc cell constants of 3.165(1), 3.152(1), 3.149(1) and 3.147(1) Å respectively. These values agree well with the expected cell constants (3.163, 3.154, 3.149 and 3.148 Å) and confirm that true alloys, rather than biphasic mixtures of Mo and W, have been formed.

It is surprising that the degree of homogeneity observed in the precipitate can be obtained by precipitating two ammonium salts which apparently contain polyanions of different stoichiometries, that is $\text{Mo}_7\text{O}_{24}^{6-}$ and $\text{W}_{12}\text{O}_{41}^{10-}$. However, a modification of sodium tungstate containing the $\text{W}_7\text{O}_{24}^{6-}$ ion has been prepared by boiling aqueous solutions of sodium paratungstate⁷ and it is possible that the initial heating step in the procedure described above results in the formation of heteropolyanions of the type $(\text{Mo}_{7-n}\text{W}_n\text{O}_{24})^{6-}$ so that a rather homogeneous distribution of the metals is frozen in before precipitation.

The alloy synthesis described here may be adapted for the preparation of a wide range of alloys containing metals which can normally be obtained by hydrogen reduction of an appropriate compound. This group comprises almost all the metallic elements to the right of molybdenum and tungsten in the Periodic Table. The method of precipitation will clearly depend on the particular elements involved and, in some cases, milder reducing conditions may be appropriate. The most exciting application of the procedure is likely to be in catalysis where the small particle size obtained in a low-temperature synthesis is particularly advantageous.

I thank Dr Michael Pope for a useful discussion, Mrs Ann Stoker for technical assistance and the SRC for grant GR/A/3320 towards an electron microscope.

Note added in proof: My coworkers and I have recently demonstrated that alloys in the systems W–Re, Pt–Ru and Fe–Ni can be prepared by related methods.

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Geometry of transform zones

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Asymmetric sea-floor formation at oceanic spreading centre/transform fault intersections is predicted by the steady-state (average) geometry of finite-width transform fault zones. In the central North Atlantic, geological and geophysical data near both small-offset (FAMOUS Fracture Zone B) and large-offset (Kane Fracture Zone) transforms support this geometry. We report here observations made on both regional and local scales which suggest predictable systematic development of asymmetric features in these areas.

Sea-floor spreading occurs along mid-ocean ridges¹. These oceanic rift zones form the continuous boundaries between diverging lithospheric plates. To a first approximation, the plates are rigid—major deformation occurs only at their boundaries². The diverging or accreting boundary between two plates consists of a string of discontinuous elongate spreading-centre segments

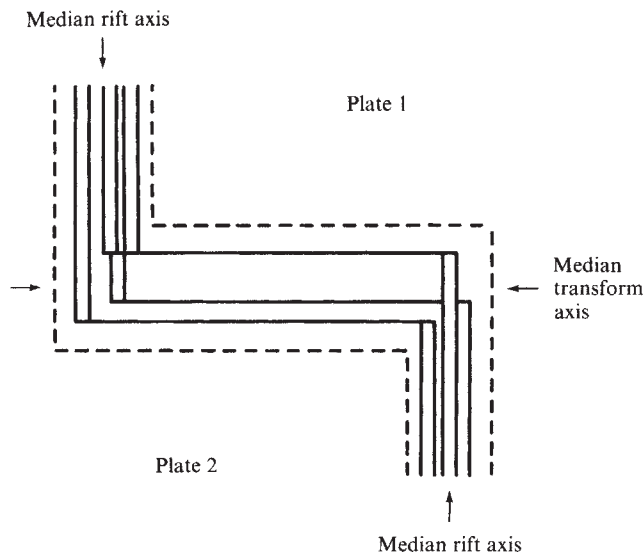


Fig. 1 Three successive transform fault events on a divergent plate boundary. The dashed lines mark the 95% width of the active plate boundary. Successive strike-slip faults and corresponding rifts (heavy double lines) are randomly distributed about their respective median axes. Half the time rifting will extend beyond the median axis of the transform fault zone. The steady-state (average) geometry of a transform zone formed by a succession of these events is shown in Fig. 2.

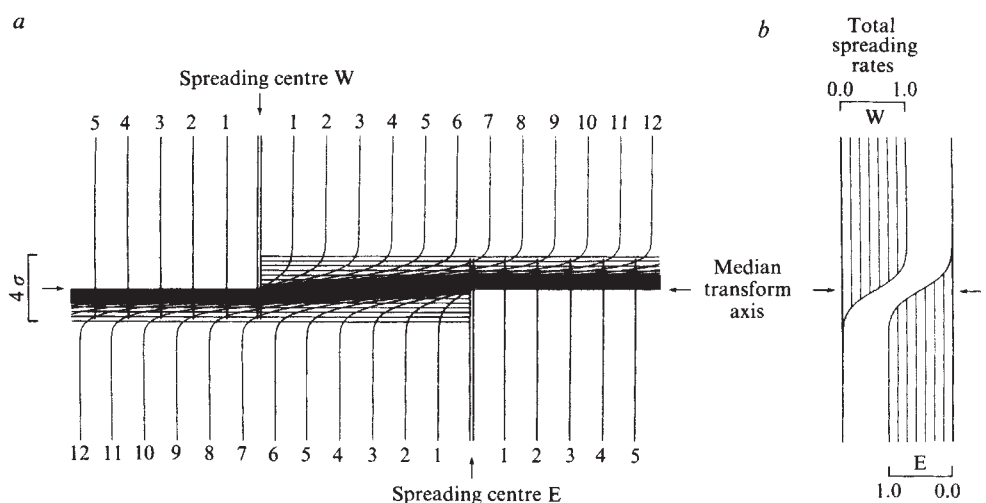
where young oceanic crust is accreted in a predominantly extensional regime. The extensional segments are joined by transform fault zones³ which represent a regime of predominant shear displacement, parallel to the direction of spreading. Spreading centres have a typical width of 2–10 km (refs 4, 5) over which most of the volcanic emplacement of oceanic crust occurs, whereas extensional tectonic deformation may be active over a broader zone⁴. Based on observations from manned submersibles and deep-towed instruments, the time-integrated width of crust affected by tectonic deformation in Atlantic transform fault zones may be as much as a few tens of kilometres^{6–8}. At a given time, however, strike-slip activity in a transform zone may be restricted to a single fault⁹ but averaged over a longer period of time (steady state), strike-slip deformation will have affected a zone of finite width (see Fig. 1).

We consider now the steady-state (average) configuration of a finite-width transform zone. Let the strike-slip activity be randomly distributed about the median axis of the transform zone. The assemblage of strike-slip faults constitute transform faults which, as Wilson³ pointed out, provide the continuity of the active plate boundary between spreading-centre segments on either side of the transform. Following Wilson's definition, strike-slip transform activity terminates in extensional activity at both ends. Thus, if transform fault deformation occurs over a zone of finite width, then, logically, half the time the rift termination will extend beyond the median axis of the transform zone (see Fig. 1).

Figure 2a shows the steady-state model of a finite-width transform zone that offsets two spreading-centre segments. The numbered isochrons illustrate the steady-state geometry and asymmetric evolution of the sea floor in a transform zone. Consecutive isochrons describe progressive stages of strike-slip deformation of the linear isochrons formed originally in the spreading centres of Fig. 2a. This geometry has been computed for strike-slip transform activity that is normally distributed about the median axis of the transform zone with standard deviation σ . Thus, 95% of the strike-slip activity occurs within 4σ , or there is a 95% probability that strike-slip activity will occur within $\pm 2\sigma$ from the median axis. The total spreading rates in the individual spreading centres are shown in Fig. 2b. The rigid-plate geometry requires that the sum of the total spreading rates in the direction of spreading is a constant everywhere on the accreting boundary. At the median transform axis, the contribution to the total spreading for both spreading-centre segments is equal. Thus, up to the median transform axis, the spreading velocity must remain constant on one side of the spreading centre (outside the active transform zone) while on the other side, spreading decreases to zero at the median axis (Fig. 2a). In other words, at the intersection of a spreading centre with a transform zone, accretion of sea floor should become progressively asymmetric until it is fully asymmetric at the median axis. Beyond the median transform axis, extension and magmatism could propagate into crust that was formed originally at the other spreading centre, resulting in mixing of young crustal material with older crust that has a transform-deformation history.

The steady-state geometry in Fig. 2a predicts that the average age of the sea floor in the transform zone will be older than that of the surrounding sea floor. The inactive trace of the transform zone will be only half the width of the active transform and will consist of mixed-age oceanic crust. Sea floor formed in the transform-zone segment of the spreading centres will have no

Fig. 2 a, Steady-state (average) geometry of a finite-width transform zone that offsets two spreading centres (heavy double lines). This geometry has been computed for strike-slip transform fault activity that is normally distributed about the median axis of the transform zone with standard deviation σ . The numbered isochrons illustrate the asymmetric evolution of the sea floor in a finite-width transform zone. Note the mixed-age isochrons in the inactive parts of the transform. Hachures indicate sea floor with transform deformation history. b, Total spreading rates in each spreading centre. Rigid-plate geometry requires that the sum of these total spreading rates is a constant everywhere along the plate boundary.



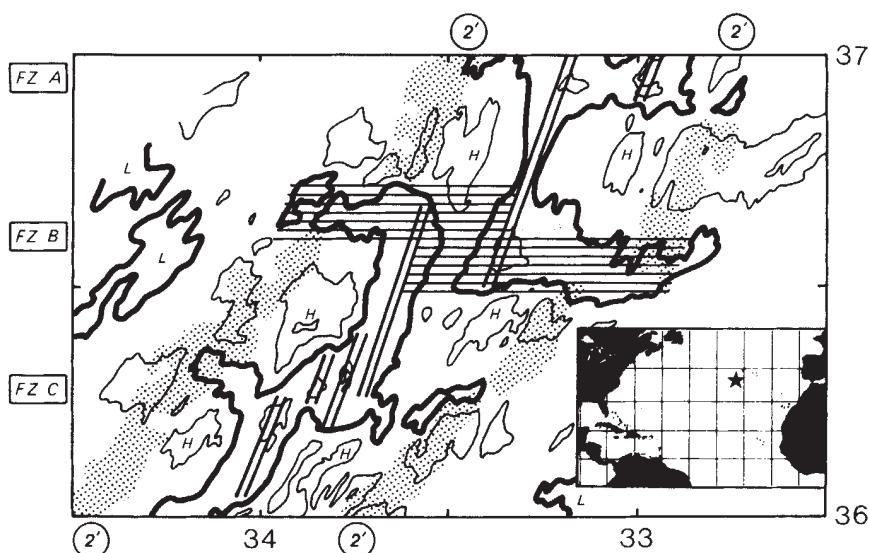


Fig. 3 Asymmetric bathymetry and marine magnetic anomalies 2' (stipples, after ref. 11) in FAMOUS Fracture Zone B. Depth contours are in fathoms. Active spreading axes (after ref. 11) are indicated by heavy double lines. The asymmetric transform zone geometry of Fracture Zone B is illustrated by a pattern of hachures explained in Fig. 2. Similar geometries can be drawn for Fracture Zones A and C.

matching counterpart on the opposite plate—the distribution of youngest volcanics at the transform intersection will be highly asymmetric and marine magnetic anomalies (isochrons) will not match.

Although this model may not be applicable to all classes of ridge-ridge transform faults, geological and geophysical data from two separate areas agree with this geometry. The first is the FAMOUS detailed survey located at latitude 36.5° N on the Mid-Atlantic Ridge crest between the Azores and the Oceanographer Fracture Zone. The bathymetry¹⁰ and segments of marine magnetic anomaly 2' (stipples)¹¹ around FAMOUS Fracture Zone B are shown in Fig. 3. The active transform section of Fracture Zone B offsets the Mid-Atlantic Ridge ~20 km in a right-lateral sense. Focal mechanism solutions of earthquakes and structural trends in the FAMOUS fracture zones suggest an east-west direction of relative plate motion in the central North Atlantic^{12,13}. We assume the east-west direction of spreading has been active since magnetic anomaly 2' (3 Myr BP). A relevant feature of the bathymetry (Fig. 3) is the

existence of parallel extensions of the adjacent spreading centres in Fracture Zone B and an asymmetric distribution of the fracture zone valleys^{11,14}. In the magnetic anomaly pattern, northwestern anomaly 2' terminates at the (steepest) northern wall of the western fracture-zone valley whereas northeastern anomaly 2' continues across the eastern fracture zone and terminates at the (steepest) southern wall of the valley.

These observations of Fracture Zone B suggest a 20-km wide transform-zone geometry (hachures in Fig. 3) that has been active during the past 3 Myr of central North Atlantic sea floor spreading. The transform-zone segment of northeastern anomaly 2' has no matching counterpart on the western plate. According to the model, young sea floor formed in the transform zone at the northern spreading centre will be transported to the east undeformed, but to the west it will be deformed in the active transform zone and then overprinted when it passes through the southern spreading centre. In this small-offset transform zone, spreading-centre activity seems to extend well beyond the median transform axis as indicated by the echelon rift valley

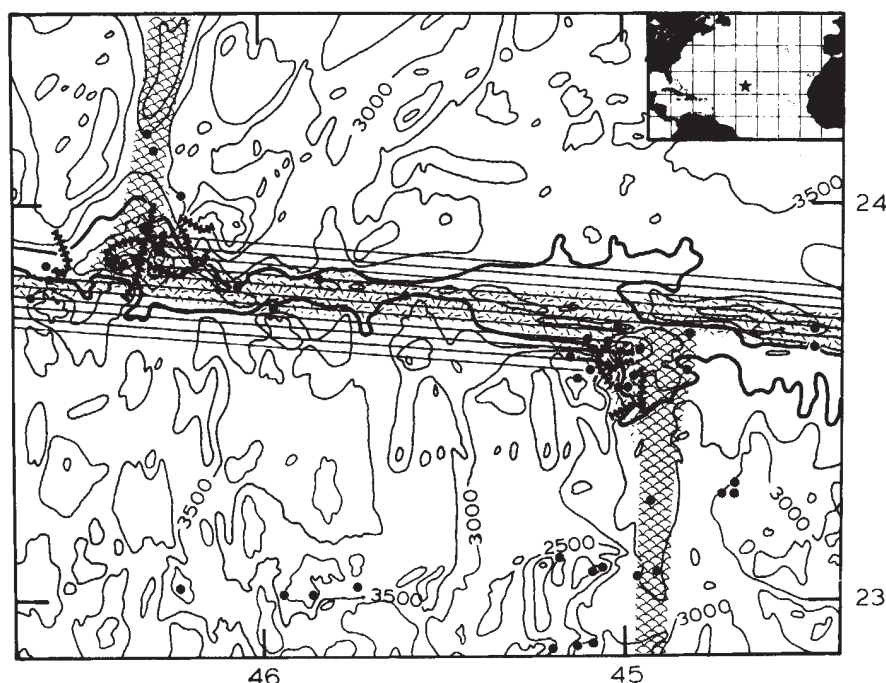


Fig. 4 Generalized geology of the Kane Fracture Zone. Bathymetric contours in metres, 500-m interval (after ref. 16). ●, Dredge haul locations; bold hachured lines, Angus camera runs (after ref. 8); scalloped pattern, young pillow basalts with negligible pelagic cover; dashed pattern, transform zone lithologies including basalt, metabasalt, greenstone, gabbro, metagabbro, serpentinite and various breccias; no pattern, areas of older basalts with variable cover of carbonate ooze. Asymmetric pattern of hachures (as in Figs 2 and 3) indicates the inferred extent of crust affected by transform tectonics.

extensions and the northeastern segment of anomaly 2'. This suggests that the physical properties of the deformed lithosphere within the transform zone at the ridge intersections are not significantly different from the physical properties of the lithosphere in spreading centres outside of transform zones and that spreading may occur in both areas.

The active transform section of the Kane Fracture Zone offsets the Mid-Atlantic Ridge approximately 160 km in a left-lateral sense near latitude 24° N (see Fig. 4)¹⁵⁻¹⁸. The fracture-zone valley is outlined by the 4,000-m contour in Fig. 4 and has a width of 10–20 km over most of its length. Combined dredging and acoustically navigated bottom camera *Angus* surveys in the fracture zone reveal an asymmetric distribution of young pillow basalts with respect to highly altered and faulted lithologies as well as the major tectonic features at the ridge intersections¹⁶.

Fresh pillow basalts with negligible pelagic cover are exposed in the median rift valley floors and at the base of the older walls in the transform zone opposite both ridge intersections (scallop pattern in Fig. 4). Fissures and faults in the young pillow lavas at the intersections indicate extension normal to the regional trend of the spreading centre⁸. Thus, sea-floor spreading seems to be continuous across the Kane Fracture Zone valley at the ridge intersections.

Pillow basalts with a variable cover of calcareous ooze occur along the median rift valley walls and marginal highs north and south of the fracture zone. Pillow basalts are also found on the floor and up the younger walls of the fracture zone outside the transform region (no pattern in Fig. 4). However, the walls of the active transform zone, the older fracture zone walls outside the transform, and oblique-trending median valley walls near the intersection provide exposures of a variety of lithologies including basalt, metabasalt, greenstone, gabbro, metagabbro, serpentinite and various breccias (dashed pattern in Fig. 4). Most of these lithologies are variably fractured and covered with ferromanganese crusts.

The asymmetric distribution of pillow basalts is best documented at the western Kane intersection where *Angus* coverage is more extensive (see Fig. 4). Relatively fresh pillow lavas extend down the fracture-zone valley a considerable distance to the west. Immediately to the east of the western intersection, the floor of the transform is largely covered with sediments and a few older appearing outcrops of brecciated rock. *Angus* coverage of transform zone lithologies immediately to the west of the eastern Kane intersection and fresh basalts dredged at the base of the older wall in the transform zone opposite the eastern ridge intersection suggests a similar asymmetry. We indicate the proposed transform zone geometry with hachures in Fig. 4. The relative abundance of plutonic and metamorphic rocks dredged from the older walls in the transform zone opposite the ridge intersections indicates little if any recent volcanism in these areas. The young volcanics in the fracture-zone valley terminate abruptly against old deformed crust that is passing out of the active Kane transform zone. In contrast to the small-offset FAMOUS Fracture Zone B, extension and magmatism do not seem to continue across the width of the large-offset Kane transform zone, but presumably terminate near the median transform axis. The age contrast across transform zones probably controls the nature and extent of the rifting that propagates into older crust at ridge-transform intersections.

In conclusion, these studies show that various asymmetric aspects of some ridge-transform intersections on the Mid-Atlantic Ridge can be explained by a steady-state geometry of transform fault zones that are a few tens of kilometres wide. The agreement between observations and steady-state model suggests that even on a 10-km scale, the process of sea-floor formation along an accreting boundary can be considered more uniform than random.

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Subduction of the Iapetus Ocean crust beneath the Møre Gneiss Region, southern Norway

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Basement-cover relations between Caledonian nappes and underlying Precambrian basement along the western coastline of southern Norway have long intrigued geologists and considerable progress has recently been made in understanding the Caledonian geology¹⁻³, geochronological studies proving particularly useful. For example, the Precambrian gneisses along the coast from Bergen to Namsos yield ages up to 1,700 Myr, supposedly the age both of the formation of the rocks and the main metamorphism and deformation phases⁴. The Lofoten-Tromsø archipelago is a presumed continuation of this basement block once sandwiched between the Caledonides of Norway and Greenland. An important phase of rock formation and metamorphism affected the gneisses here at around 1,800 Myr, whereas subsequent orogenies at 1,100 and 400 Myr (Grenville-Sveco-Norwegian or Dalslandian, and late Caledonian orogenies, respectively) had little effect. COCORP reflection profiling in the southern Appalachians⁵ has demonstrated that crystalline 'basement' has been thrust for a large distance over autochthonous early Palaeozoic sediments of the proto-Atlantic continental margin. Data from a seismic profile⁶ across the Møre Gneiss Region (often called the northwestern basal gneisses of southern Norway) show evidence of P-wave velocity reversal at a depth of about 14 km, and the associated low-velocity layer (LVL) has a thickness of 4 km. As there is no excess of heat flow to account for the LVL, we consider here the hypothesis that oceanic crust and accompanying sediments have been subducted under the gneisses.

The refraction-wide-angle reflection seismic profile is indicated in Fig. 1, together with the two shot-point locations I and II. Five 3-component seismometers were moved along the profile at an average spacing of 2.5 km, while explosive charges of 25–300 kg were fired in water depths of ~20 m at the shot points. Altogether 145 seismic field records were obtained, but many of them were not suitable for detailed digital analysis, although several poorer-quality records were used for extracting first arrival travel times.

After the necessary elevation corrections had been made, a Herglotz-Wiechert inversion procedure was applied to the first

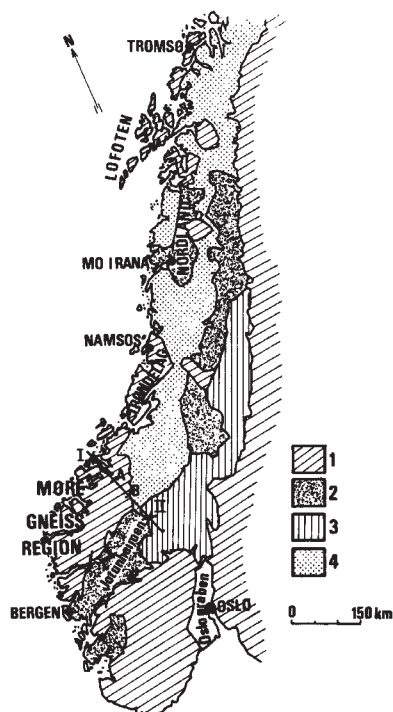


Fig. 1 Simplified tectonic map of Norway redrawn after Reymer⁷, showing shot points I and II of the seismic profile crossing the Møre Gneiss Region. 1, Precambrian basement, including the generally presumed autochthonous Møre Gneiss Region, with radiometric ages in the range 1,900–1,200 Myr; 2, mostly Precambrian nappes (1,200–850 Myr) also including structures of Caledonian ages; 3, Lower Palaeozoic elements, (par)-autochthonous-allochthonous structures of mostly Cambrian–Silurian ages; 4, Lower Palaeozoic elements, typical nappe substructures having a presumed south-eastern direction of thrusting.

arrival observations (see Fig. 2a). The continuously increasing velocity distribution obtained (Fig. 2b) was truncated at 12–14-km depth, because the pronounced offset in the travel-time curves suggests the existence of a LVL in the upper crust. Note that strong lateral inhomogeneity effects are ruled out by detailed comparison of records from the two shot points.

Modelling of the lower crust is relatively uncertain in view of a maximum shot-detector separation of 190 km. The outstanding feature, however, of the velocity model obtained is the roughly 4-km thick LVL in the upper crust. Because of objections to such a feature on the grounds of both realistic heat-generation mechanisms and limited resolving power of our data, we have undertaken extensive synthetic seismogram analyses. These experiments demonstrate that the most appropriate model for reconciling discontinuous travel-time data and amplitude distributions of primary and secondary phases involves the introduction of a LVL, which can generally be recognized best from reflections from the top of it. Characteristics of these arrivals are: (1) a 180° phase shift relative to the first arrivals; (2) the travel time and apparent velocity; and (3) absence of associated head-wave arrivals⁸. Figure 2c shows an extract from both the observed record section (shot point I) and a synthetic section computed from the model in Fig. 2b, covering the distance interval over which the LVL reflections are observed. The upper-crustal first arrivals (d) and the reflections from the top and bottom (e and f respectively) of the LVL are well modelled by the synthetic section.

From our data it is impossible to estimate accurately the actual horizontal extent of the LVL, even in the direction of the profile. Our modelling experiment here demonstrates that, even if the LVL were present under the entire profile, the reflections would

be observable only where they are actually seen in the record section. However, a minimum extent of the LVL can be estimated and is indicated by AB in Fig. 1. The data also suggest that the LVL is rather flat-lying. The two velocity–depth curves of Fig. 2b can be taken to be representative at points A (curve I) and B (curve II) of Fig. 1, some 50-km apart, yielding a westward dip of the upper LVL interface of <2°.

The nature and significance of low-velocity zones are still not well understood. Extensive laboratory experiments have been undertaken to investigate the possibility of crustal velocity reversals. The main prerequisite for such phenomena is either anomalously high temperatures and/or profound compositional changes. Typical examples are areas of exceptionally high heat flow such as rift zones and areas, where high-velocity crystalline rocks have been thrust over 'soft' material of lower velocity^{9,10}. Within the Møre gneisses there is no evidence of high heat flow¹¹, so the postulated LVL must be associated with compositional changes. Further to the north near Mo i Rana, a trans-Scandinavian profile¹² also gives evidence of an upper-crustal velocity reversal under the Caledonides only (see Fig. 1).

We suggest that the crustal structure in the Møre Gneiss Region is explained by a doubling of the crust, which occurred during the final continent–continent collision of the Caledonides towards the end of the Silurian. As low-velocity rocks, sandwiched between continental rocks from two opposing continents, two types must be considered: (1) serpentinites, and (2) eogeosynclinal sedimentary rocks of relatively low metamorphic grade. It is impossible to discriminate between types (1) and (2) from the geophysical data only. Type (1) is, however, discarded because of the geological improbability of having serpentinite from the lower oceanic crust as a surface layer on top of the lower continental mass. Logically the

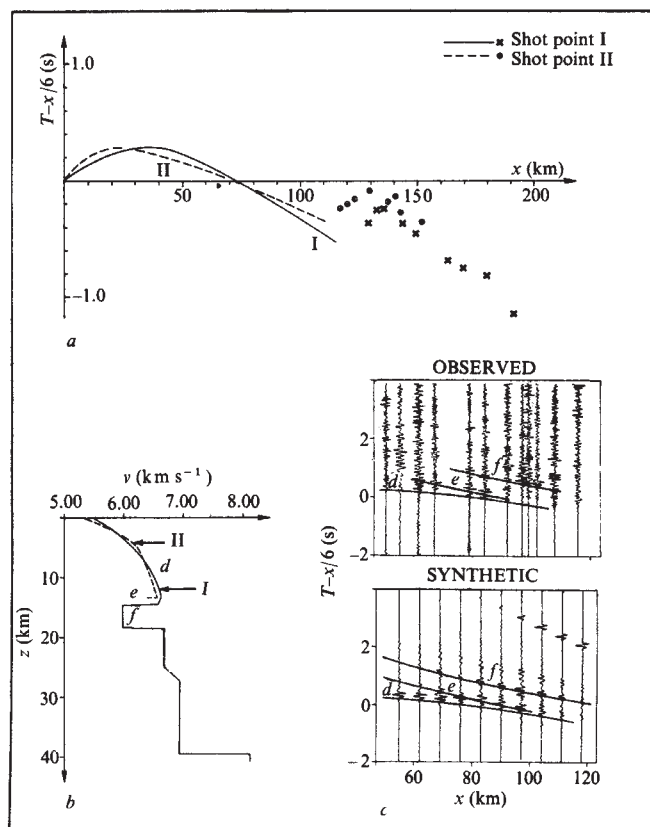


Fig. 2 a, First arrival travel times for both shot points. The continuous curves represent a slight smoothing of travel-time observations. b, Velocity–depth distribution derived from shot point I data. Dashed curve, shot point II data. c, Detail of record section (top) for shot point I and corresponding synthetic section (bottom). Selected segments of the travel-time curves as computed from the model of b (curve I) are inserted.

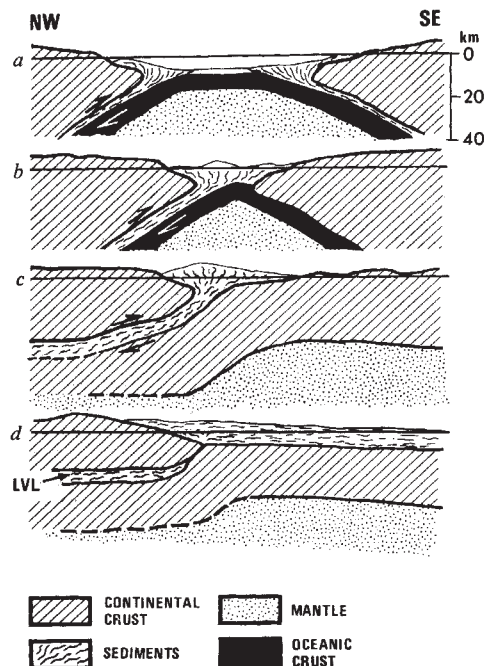


Fig. 3 Simplified cross-sections through the Møre Gneiss Region, with the closing of the Iapetus Ocean, illustrating our hypothesis for the LVL. *a*, Late stage in the closing of the Iapetus Ocean. *b*, Situation before the continents collide, with a westerly dipping subduction zone along which orogenic sediments are dragged down. *c*, The continental collision resulting in doubling of crustal thickness with sediments constituting the LVL sandwiched between the two crustal blocks. *d*, A westerly Himalayan-type mountain chain resulting from isostatic uplift, off which the nappes glide to the east.

incorporation of eogeosynclinal sediments during subduction should occur during a subduction process¹³. This hypothesis contrasts with the current thinking of many geologists working with the basement-cover relations of the Caledonides in southern Norway, who favour the view that the Møre gneisses represent an autochthonous area above which Caledonian nappes to the east have been thrust from a westerly location in the Lower Palaeozoic proto-Atlantic (Iapetus) ocean^{4,14}.

Our hypothesis relies on the general features of the Bergen-Namsos area, which exhibits a lithological and structural development clearly different from that of southeastern Norway³. This area may either be classed as a separate Precambrian block¹⁵, or may represent an overthrusting part of the American block over the Eurasian one. In the latter case, a last deformation of the Precambrian gneisses has to be of Caledonian age. This possibility is permitted by a recent Sm/Nd age study¹⁶ of eclogite lenses located in granitic gneisses in the coastal Møre area, which are found to be late Ordovician-Silurian.

A mechanism envisaging a Himalayan-type doubling in thickness of the crust of the mid-Scandinavian Caledonides is sketched in Fig. 3, based on recent suggestions for the Grenville collision¹⁷ and the Sunda trench subduction¹³. The allochthonous overthrust block of the Møre gneisses may not be restricted to the Bergen-Namsos area, but may include the basement of the whole Nordland coast as well as the Precambrian of the Lofoten-Tromsø coastal area. The buoyancy of the doubled continental crust would have produced a Himalayan-type mountain chain, off which slid the soft Cambro-Silurian orogenic rocks as nappes travelling far to the east—250–500 km, possibly even further^{18,19}. The weathered core of this chain now exhibits the eclogites. The postulated collision zone to the east of the Møre Gneiss Region (the 'Jotunheimen suture') is supported by recent detailed geological field work²⁰. Also, a recent Fennoscandian study²¹ supports the idea of a relatively thick crust (~40 km) in the coastal areas of

western and northern Norway, as opposed to southeastern Norway (Oslofjord), where the Moho depth is only around 30 km. Finally, some features of our model have earlier been hinted at³ and also used to explain the surprisingly young age of the Møre eclogites¹⁶.

Although our interpretations of the LVL beneath the Møre Gneiss Region may be considered premature, we conclude that the identification and interpretation of similar crustal velocity reversals will have an important application to the problem of unravelling continental collisions and locating the sutures between them.

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Guidance system used in moth sex attraction

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It has been proposed^{1–4} that a flying male moth finds its way to a 'calling' female or other small, distant source of wind-borne sex pheromone using two contrasting anemotactic manoeuvres: (1) upwind flight in response to the onset or increase of the pheromone stimulus, maintained as long as the stimulus continues, and (2) cross-wind flight with switching between left and right of the wind line (the so-called 'track reversals'⁴ or 'tack reversals'⁵ seen in casting or zig-zagging) which occurs only in response to the loss or decrease of the pheromone stimulus. A simpler system can now be proposed in the light of wind-tunnel experiments on male summerfruit tortrix moths (*Adoxophyes orana*) entering homogeneous pheromone clouds. The moths did not fly persistently upwind with continuous pheromone stimulation, and their programmed left-right track reversals occurred in response to pheromone onset, not loss, and continued after pheromone loss but with widening cross-wind excursions between reversals.

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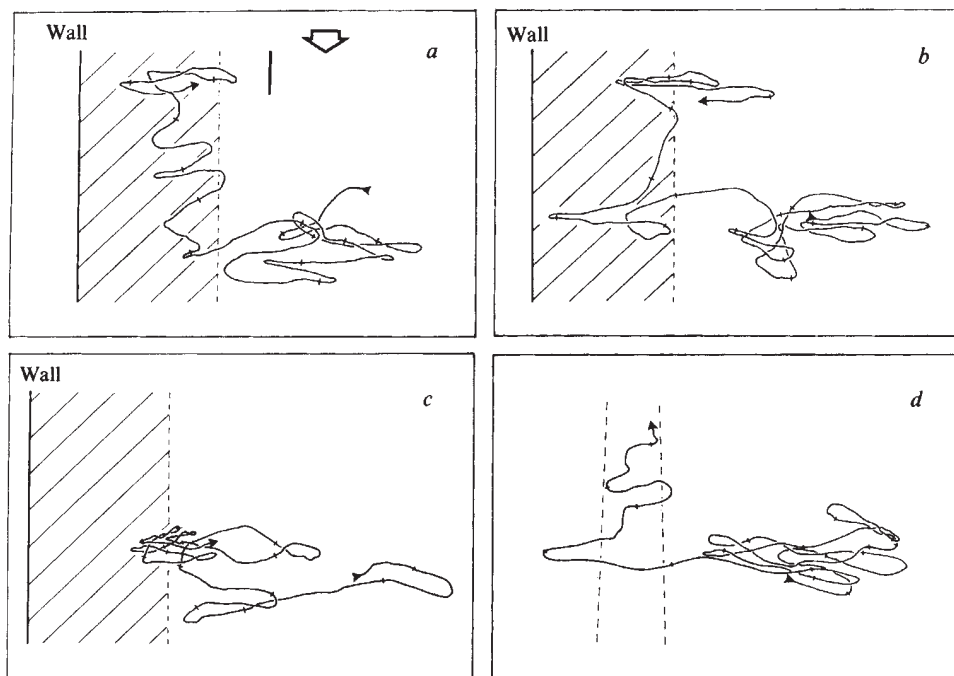


Fig. 1 Samples of flight tracks in the wind tunnel. Time marks at 0.5 s intervals. 0.1-m scale bar and wind direction arrowhead in *a* apply to all tracks. *a, b*, Cross-wind casting after removal of a pheromone plume, leading to entry into the pheromone corridor (hatched) and a brief upwind advance, followed by resumed casting. *c*, Same as above but entry into the pheromone corridor followed only by narrowed casting without upwind progress. *d*, A normal, structured, pheromone plume (broken lines indicate its smoke-simulated, time-averaged outer envelope) added to a pheromone cloud filling the entire tunnel—on encountering this plume the casting moth 'locks-on' to it and advances upwind along it as if the plume were in clean air.

The male moths were held in constant light without females for 3–4 days from emergence and then in darkness for ~2 h before transfer to the wind-tunnel⁴ (working section $1.9 \times 1.2 \times 0.45$ m) in ~12 lx from overhead fluorescent lamps and a smooth air flow of $\sim 0.24 \text{ m s}^{-1}$. The pheromone was not presented to the flying moths in the normal form of a broken, filamentous plume because it is then impossible to say when the flier is and is not meeting pheromone, and stimulus-response relations cannot be identified. In these experiments, therefore, the pheromone was presented as a homogeneous cloud. The pheromone cloud filled a floor-to-ceiling 'corridor' about 0.36 m wide against one wall of the tunnel, and smoke simulation showed a sharp boundary with negligible mixing between this 'corridor' air and the contiguous body of clean air moving in parallel down the rest of the tunnel.

The following procedure was used to select only responsive moths and to ensure they received the pheromone test-stimulus (by entering the corridor) in a standard and observable manner. Each moth was first exposed to the normal type of plume from a small pellet of polyvinyl chloride containing synthetic pheromone (a 9:1 mixture of *trans*- and *cis*-11-tetradecenyl acetate) in the clean part of the tunnel. Moths were discarded unless they responded by taking off, 'locking-on' to the plume (that is, heading into wind and restricting their cross-wind movements to the immediate vicinity of the plume) and then advancing up it for more than 1 m, keeping away from the side corridor of diffuse pheromone. Each moth that responded in this way was then induced, by suddenly removing the pheromone plume, to stop advancing upwind and to cast across wind increasingly widely^{2,4} and so enter the side pheromone corridor within a few seconds. A control set of moths was treated in the same way in the absence of pheromone corridor. The horizontal component of the flier's movements was recorded on videotape, and the position and time of each track reversal (switching from one side of the wind-line to the other) were later read to provide data on the cross-wind extent, duration and net direction of each inter-reversal leg of the track. The tracks were also traced frame by frame to determine the incidence of upwind advances. For this purpose an upwind advance was defined as a section of the flight track that continued with no downwind movement at all until upwind progress of at least 0.2 m had been made. Such advances often comprised zig-zags (Fig. 1*a*) but without the track deviating from due upwind by more than 90°.

Twenty-nine moths were recorded with the side pheromone corridor present. They flew in and out of it repeatedly and 40 of

their entries were made after they had spent more than 2 s outside it. 35% (14) of these entries were followed directly by an upwind advance as defined, as against 5% (5) of 93 equivalent legs in the control series without any pheromone cloud ($P < 0.001$). The time from crossing the border into the pheromone corridor to the next track reversal averaged about 0.2 s, and an upwind turn was already evident in the mean angle to wind of the 40 legs starting then. Taking due upwind as 0°, this angle was 70.0 ± 4.2 (30% of legs at $< 60^\circ$) as against $87.0 \pm 1.1^\circ$ (all legs $> 60^\circ$) in the controls. By contrast, there was no significant upwind response when the moths entered the pheromone corridor after spending less than 2 s outside it. Only 2% (7) of these 438 entries were followed by an upwind advance, significantly less than the figure of 35% after more than 2 s outside ($P < 0.001$).

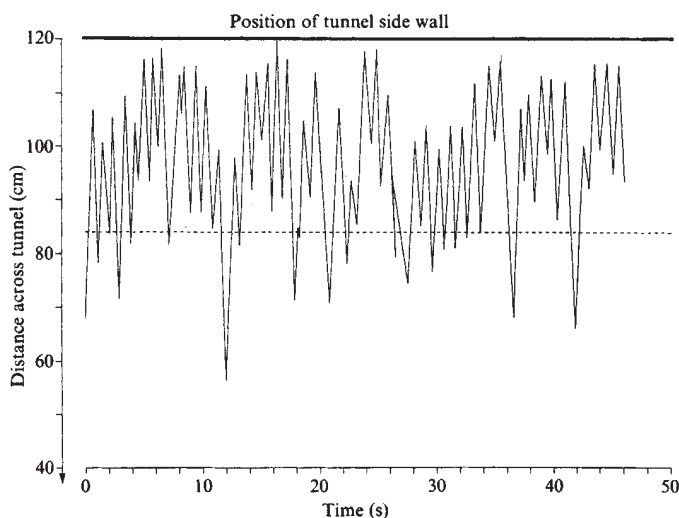


Fig. 2 The cross-wind component of one moth's flight track in the wind tunnel, plotted against time. Only the positions at which the track reversed across wind were measured, the straight lines joining these reversal points being interpolations. The broken line marks the approximate position of the border between the 'clean' part of the airflow and the pheromone 'corridor' extending to the tunnel wall at 120 cm. Note frequent track reversals inside the pheromone corridor, which cannot be attributed to any change in pheromone stimulation.

The lack of response on re-entering the pheromone after less than 2 s outside it implies that the moths were then adapted (*sensu lato*) to the given pheromone stimulus, at least in respect of the upwind flight response, and that substantial disadaptation required more than 2 s in clean air. A second indication of adaptation was that the upwind advances were mostly brief, of the order of 1 s, the moth then reverting to cross-wind casting and 'keeping station' against the wind as in Fig. 1a and b. This arrest of upwind progress could not be attributed entirely to a high pheromone concentration in the corridor although high concentrations are known to retard upwind flight along pheromone plumes⁴⁻⁶. These same moths had all progressed freely up the broken plume from the single-pellet pheromone source shortly before, and the pheromone concentration in the individual filaments of the plume will have been higher than in the corridor cloud. In a parallel treatment, 26 moths, casting widely after plume removal as before, were engulfed by a wind-borne, homogeneous pheromone cloud that was not confined to a side corridor but filled the entire tunnel. Again there were brief upwind advances with reversion to cross-wind casting, although the cloud concentration was at least 30 times less than the mean concentration in the plume up which these same moths had progressed freely just before. Furthermore on several occasions when moths had been casting and making no progress for many seconds within such a ubiquitous pheromone cloud, they were also presented with a normal, broken, pheromone plume from a single pellet. On encountering this superimposed plume the moths promptly 'locked on' to it and advanced up it in zig-zags (Fig. 1d) in the same manner as they had done when presented with the same plume in otherwise clean air. Evidently the moths were adapted to the pheromone concentration in the cloud but still fully responsive to increases above that level.

Entries into the side pheromone corridor, or the ubiquitous pheromone cloud, led not only to some upwind advances but also, and much more predictably, to a restriction of cross-wind movement. This was not mainly due to turning upwind although that, of course, enhanced the effect. When combined with an upwind advance, the restriction produced narrow upwind zig-zagging as in Fig. 1a. More frequent was a simple narrowing of the cross-wind casting already in train, without upwind progress, as in Fig. 1c. The narrowing was due mainly to a reduction of flight speed rather than time interval between track reversals, and it persisted for at least 2 s in clean air after emergence from the pheromone corridor. As a result of this persistent, pheromone-induced narrowing the moths' movements became concentrated around the free border of the pheromone corridor and often crossed it in both directions (Figs 1a-c, 2). Thus there were many instances of turning back into the pheromone corridor after leaving it, but there were also many cases of two or more consecutive track reversals entirely inside the pheromone corridor (Figs 1a, 2). This contradicts the above theory that track reversal is a response to losing the scent. There were a total of 224 cases of moths moving across the inside of the pheromone corridor towards its free border with the clean air, but then turning back well before reaching the border. Furthermore the 26 moths engulfed in the ubiquitous pheromone cloud already mentioned made an average of 27 (3-59) consecutive track reversals each, all without any pheromone decrease.

We conclude that persistent upwind flight requires decreases as well as increases of the pheromone stimulus and that the programmed switching of the flight track between left and right of the wind-line, seen in zig-zagging and casting flight, is not a response to loss of pheromone. It is initiated by pheromone, and then modulated by pheromone changes, the amplitude of these cross-wind movements changing inversely with the strength of the pheromone stimulus. This would provide a simpler guidance system than those previously proposed.

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Environmentally dependent sensitive periods for avian vocal learning

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Experience during brief periods of development can exert a profound influence on later life¹. Among songbirds, experimental evidence for enhanced vocal learning during a relatively brief period early in life is well documented^{2,3}. The timing of vocal learning with respect to dispersal is fundamental to our understanding of many population processes, yet the significance of the sensitive period remains unclear. We report here that in our study of the marsh wren (*Cistothorus palustris*), a North American songbird, we found that the sensitive period for song learning is not rigidly programmed with respect to dispersal and/or migration; two environmental factors, the photoperiod and the amount of adult song heard during the hatching year, influence the nature of the sensitive period during the hatching year, the ability to learn further the next spring, and the relative dates at which young males develop their adult songs.

In our Hudson River marsh, nestling wrens hatch from mid-June to late August. Young hatched early in the year hear many adult songs and experience long days through June and July, and two previous laboratory experiments had indicated that song learning in these conditions seemed restricted to the first 60-80 days of life⁴. Young hatched late in the year, however, hear few if any adult songs⁵ and experience relatively short days during the first couple of months. If these late-hatched young are to sing and breed the next year, their learning period must also be delayed until the following spring.

To test the possible effects of the photoperiod and exposure to song on the timing of the sensitive period, during early July we collected 18 nestling males from our Hudson River marsh at 41° north latitude. These were placed in sound isolation chambers; 10 males (hereafter called June males) were placed on a photoperiod simulating an early June hatching, whereas the remaining 8 males (August males) were on a photoperiod simulating an early August hatching. Thus, mean ages for the two groups were the same, and subsequent changes in daylength for each photoperiod group matched that of the home latitude. Each photoperiod group was further subdivided into three experience treatments: from roughly days 20 to 100 (Fig. 1), some males were tutored with two different song types every 5 days (a total of 32 song types; three June and two August males), some with one song type every 5 days (16 song types, four June and four August males), and some heard no songs during their hatching year (three June and two August males). During development of their adult songs the next spring all 18 males were exposed daily to 12 additional song types.

The physiological effects of the two photoperiod treatments were substantial (Fig. 1). Median ages for onset and termination of moult in the August males were 45 and 71 days compared with 62.5 and 95 days for the June males. Early morning practice singing (subsinging) also revealed that the autumn period of

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subsong was sharply curtailed in the August males; thus, between days 70 and 140, 72-min recordings from each male revealed subsong for a median period of only 11 min for August males but 54 min for June males. The quality of this subsong during the autumn period differed substantially between groups: the August males never passed a very rudimentary rambling characteristic of the very earliest stages of song development, but many of the June males developed more advanced and stable songs. Finally, the 'migratory restlessness' of the August males began and ended much earlier than that of the June males.

Despite the photoperiodic effects on moult, subsong and migratory activity, the sensitive periods for vocal learning during the autumn did not differ significantly between the two groups; June males seemed to learn⁴ more songs and learn them at a later age than August males ($P=0.1$ in both cases; 1-tailed Mann-Whitney U -test). August males acquired appreciable repertoire sizes in daylengths when songs in the wild would not normally be available to them. There was a significant difference the next spring, however, as four of the eight August males

imitated songs from the spring training tapes, whereas none of the 10 June males did; the probability of all four of the spring imitators being August males by chance alone is only 0.023.

Those four August males which learned spring songs included two males with no autumn tutoring and two males hearing only one song every 5 days during autumn. The spring learners tended to be the August males which had acquired the fewest imitations during their hatching year ($P=0.06$). In addition, in both photoperiod treatment groups, those males experiencing no songs during their hatching year were the last to develop their final songs the next spring ($P=0.018$ and 0.05 for June and August males, respectively), indicating that lack of exposure to songs can actually delay song development.

Experience with tutor songs during the autumn affected two other features of vocal learning; first, the autumn sensitive period was extended in males hearing fewer songs, as males hearing only one song every 5 days acquired their autumn imitations at a slower rate than the males hearing two songs every 5 days. By day 60, there was a significant difference in the fraction of the final repertoire which had been learned ($P=0.036$). Second, experience played an important part in the quality of the songs developed by the males. The three June males experiencing no songs during their hatching year improvised a total of 60 highly abnormal song types not recognizable as marsh wren songs, whereas the June male imitating the largest number (13) of songs from the training tapes developed only one non-imitation. Song repertoire sizes did not differ significantly among treatment groups, and the number of imitated songs and improvised (therefore usually highly abnormal) songs in the males' repertoires were negatively correlated ($P<0.01$).

Thus, the sensitive period for song learning in the marsh wren is not rigidly age dependent but to some extent environmentally determined. The degree of exposure to adult songs during the hatching year influences the timing of song learning, but this opportunistic vocal learning seems constrained by physiological

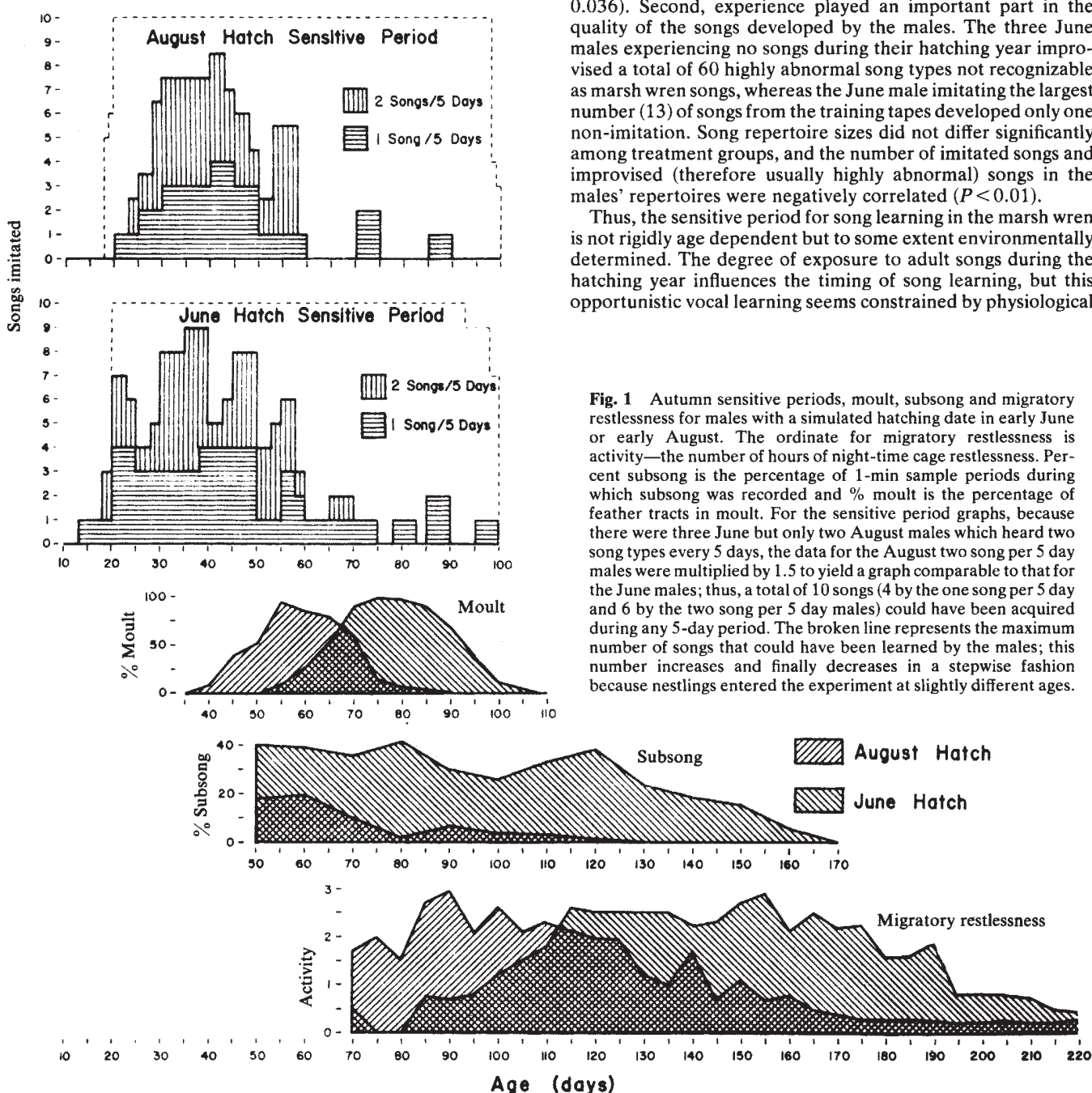


Fig. 1 Autumn sensitive periods, moult, subsong and migratory restlessness for males with a simulated hatching date in early June or early August. The ordinate for migratory restlessness is activity—the number of hours of night-time cage restlessness. Percent subsong is the percentage of 1-min sample periods during which subsong was recorded and % moult is the percentage of feather tracts in moult. For the sensitive period graphs, because there were three June but only two August males which heard two song types every 5 days, the data for the August two song per 5 day males were multiplied by 1.5 to yield a graph comparable to that for the June males; thus, a total of 10 songs (4 by the one song per 5 day and 6 by the two song per 5 day males) could have been acquired during any 5-day period. The broken line represents the maximum number of songs that could have been learned by the males; this number increases and finally decreases in a stepwise fashion because nestlings entered the experiment at slightly different ages.

consequences of the photoperiod; irrespective of early song treatment, only males which were raised under daylengths simulating a late season (August) hatching learned songs the next spring. Nottebohm⁶ may have simulated this late season effect to an extreme degree when he castrated a young chaffinch (*Fringilla coelebs*) and delayed the sensitive period for song learning until administration of exogenous testosterone. Thus, in late hatching males low levels of testosterone and/or reduced singing, controlled by the photoperiod, are undoubtedly important factors in delaying the sensitive period.

Among songbird populations, late-hatched young necessarily hear fewer adult songs during the hatching year⁵ and often disperse further to the first breeding territory than the early hatched young⁷. A sensitive period which is in part environmentally determined will allow the dispersing juvenile some flexibility in when and where it learns a local song dialect and establishes its territory. Marsh wren juveniles can disperse over considerable distances⁸, and the timing of vocal learning has undoubtedly co-evolved not only with juvenile dispersal strategies but also with adult site-fidelity and habitat stability, large song repertoires, the highly complex male-male vocal interactions^{9,10} and other life-history parameters of marsh wren breeding populations. These parameters vary between populations, both within and among species^{11,12}, and will, through natural selection, influence the nature of the sensitive period for song learning.

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Sensory maps in the claustrum of the cat

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The claustrum is a telencephalic cell group (Fig. 1A, B) possessing widespread reciprocal connections with the neocortex¹⁻⁵. In this regard, it bears a unique and striking resemblance to the thalamus. We have now examined the anatomical ordering of pathways linking the claustrum with sensory areas of the cat neocortex and, in parallel electrophysiological experiments, have studied the functional organization of claustral sensory zones so identified. Our findings indicate that there are discrete visual and somatosensory subdivisions in the claustrum interconnected with the corresponding primary sensory areas of the neocortex and that the respective zones contain orderly retinotopic and somatotopic maps. A third claustral region receiving fibre projections from the auditory cortex in or near area Ep was found to contain neurones responsive to auditory stimulation. We conclude that loops connecting sensory areas of the neocortex with satellite zones in the claustrum contribute to the early processing of exteroceptive information by the forebrain.

Areas 17, 18 and 19 of visual cortex are known to have overlapping fibre connections with a small dorsocaudal sector of the claustrum⁶ and the pathway from areas 17 has a rostrocaudal topography⁵. To analyse the claustral origins and terminations of these visual pathways in detail, we carried out neuroanatomical experiments with the tracers horseradish peroxidase⁷

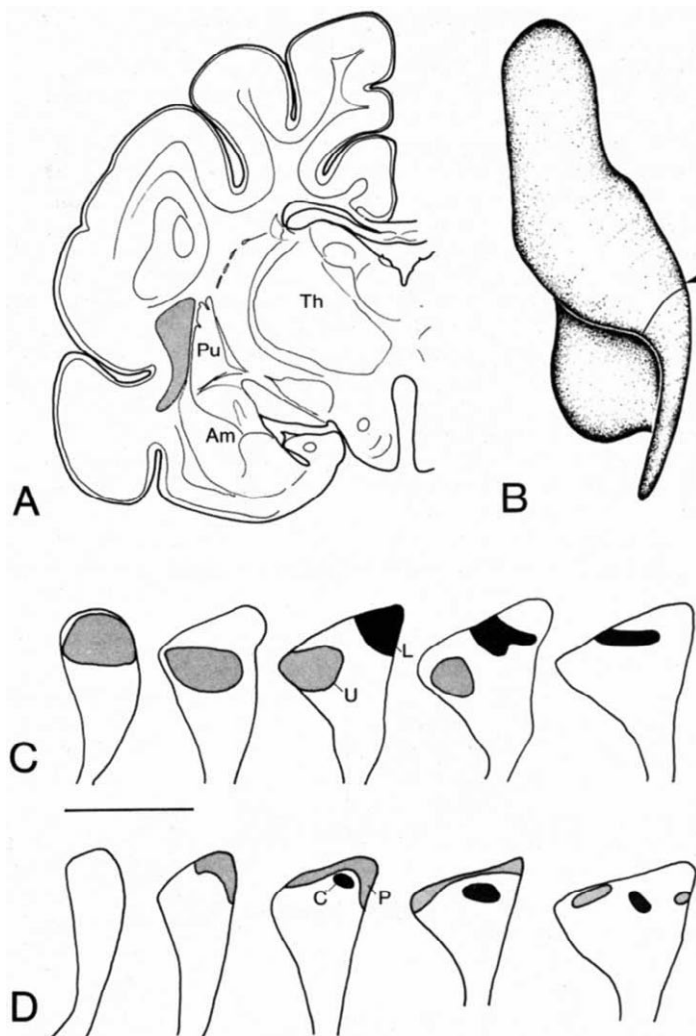


Fig. 1 A, Representative frontal section through left hemisphere ~2 mm ahead of the caudal tip of the claustrum. The claustrum is set off by cross-hatching. Am: amygdala. Pu: putamen. Th: thalamus. B, Three-dimensional reconstruction of left claustrum as viewed from a point above, behind and lateral to the structure. Total rostrocaudal extent is ~12 mm. Arrow indicates transverse level of section shown in A. C, D, Patterns of tracer labelling in the claustrum elicited by injections of HRP and NY in parts of area 17 representing vertically separated (U = upper, L = lower) and horizontally separated (C = central, P = peripheral) areas in the visual field. Transverse sections are shown at 0.5-mm intervals beginning 0.75 mm ahead of caudal tip (lateral is to the left). Scale bar, 2 mm. In each cat, HRP (25% solution in saline, 0.2 μ l) and NY (10% suspension in saline, 0.2 μ l) were injected into separate parts of area 17, with some encroachment into retinotopically overlapping parts of area 18. The brains were fixed by perfusion with 10% formalin, 0.25% glutaraldehyde and 3% sucrose in 0.1 M phosphate buffer and cut at 50 μ m. Serial sections were processed for HRP by the TMB method⁷. Retrograde and anterograde HRP-labelling was charted under dark- and light-field illumination and nuclei labelled with NY were identified by intense fluorescence exhibited under 360 nm illumination. The part of the area 17 contralateral visual-field map covered by each injection was estimated from the distribution of label in the lateral geniculate body as follows¹⁷: C, Dark stipple: HRP: vertical -15° to -40°; horizontal 0° to 30°. Light stipple: NY: vertical +5° to 0°; horizontal 0° to 10°. D, Dark stipple: HRP: vertical -5° to 15°; horizontal 0° to 10°. Light stipple: vertical -5° to -20°; horizontal 30° to 60°.

(HRP) and nuclear yellow⁸ (NY), and with isotopically labelled amino acids⁹. In 11 cats, distinguishable tracers were deposited in separate cortical sites under electrophysical guidance. This approach allowed us, in single animals, to compare patterns of claustral uptake elicited by injections in retinotopically identical parts of two areas or in retinotopically distinct parts of the same area. The findings suggest that projections to and from areas 17 and 18 are organized retinotopically and in register, with the upper-to-lower axis of the visual field represented caudorostrally in the claustrum and the central-to-peripheral axis

represented ventrodorsally (Fig. 1C, D). The same pattern is observed in cases (not illustrated) of injections involving area 19. The ventral core of the claustrum, related to the midline of the visual field, is exceptional in having connections with visual cortical areas in both hemispheres (Fig. 2D).

Guided by these anatomical findings, we performed microelectrode-recording experiments in the caudal claustrum of eight cats. Our results demonstrate the existence of a circumscribed zone of visually responsive cells closely co-extensive with the region delineated anatomically. Units and unit clusters were exclusively visual, responded best to moving bars and spots and had receptive fields ranging in area from a few degrees square to a few hundred degrees square. Most cells could be driven through either eye and some seemed to be broadly tuned for bar orientation or axis of motion. Responses were weaker and more variable than in area 17 of the same preparation. There was clear evidence for detailed retinotopy in this zone. As shown in Figs 2A, B and 3A, B, receptive fields moved downward systematically as the electrode was moved rostrally and towards the midline as the electrode was advanced ventrally.

To test the generality of the finding of topographic sensory mapping in the claustrum, we studied the slightly more rostral claustral region interconnected with primary somatosensory cortex, SI¹⁰. Restricted deposits of tracers placed under electrophysiological guidance in parts of SI representing caudal and rostral body parts labelled, respectively, dorsal and ventral sectors in this claustral zone. The pattern of connections was analysed only with respect to the longitudinal body axis because the circumferential axes of the body are not simply represented

in SI¹⁰. In electrophysiological experiments, we encountered, throughout this region, neurones with large somatosensory receptive fields occupying parts of the contralateral body (Fig. 3). All could be driven effectively by firm pressure or sharp taps and some also responded to light cutaneous stimulation. During downward traverses, receptive fields shifted gradually from the hindparts to the face (Figs 2C, 3C). This pattern of organization, though not noted in previous physiological studies¹¹, is in accord with the demonstration of somatotopy based on the anatomy (Fig. 2E).

In contrast to the findings for visual and somatosensory cortex, injections of HRP confined to the primary auditory cortex have not, in our hands, consistently produced labelling in the claustrum (compare with ref. 4). However, injections encroaching on or centred in area Ep of the posterior ectosylvian gyrus, a region of known auditory affiliations^{12,13}, have produced anterograde and retrograde labelling of a claustral zone lying beneath the somatosensory division and approximately co-extensive with it rostrocaudally. Guided by this observation, we searched for and found a region of exclusive auditory responsiveness in the claustrum. During downward passes of the electrode, this region typically was encountered within a few 100 μ m of the most ventral somatosensory activity, and was lost again within 100–200 μ m (Fig. 3, C11). Cells recorded at this depth characteristically responded to clicks or tone bursts delivered to either ear, as well as to a variety of free-field stimuli. We have not mapped the auditory zone through its full extent.

The connections of the neocortex with the claustrum are organized not only by cortical area but also by cortical layer. In

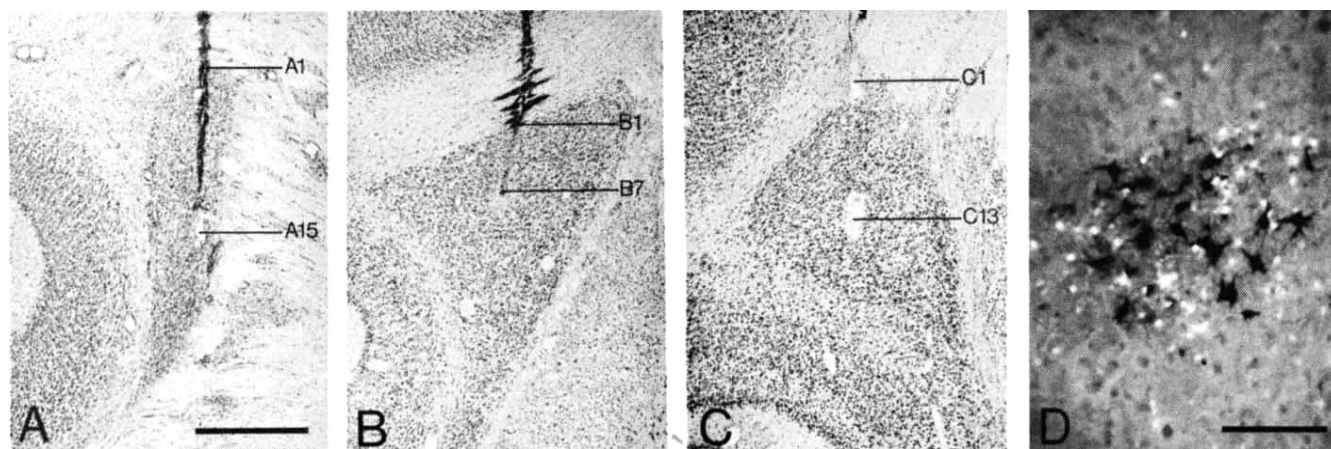
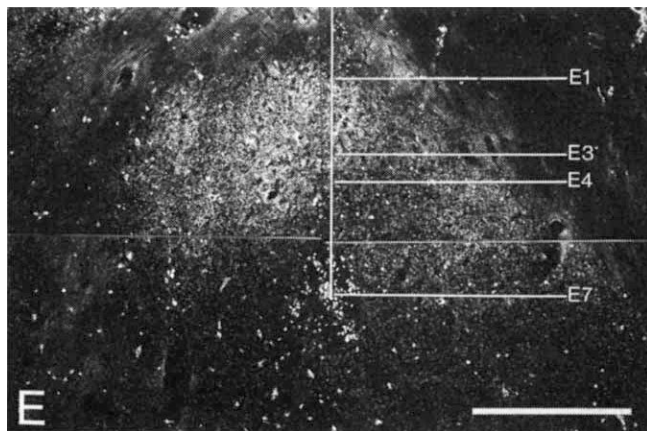


Fig. 2 A–C, Transverse sections stained by the Nissl method illustrating vertical electrode penetrations through the claustrum. Along tracks shown in A and B, the units recorded had the visual receptive fields plotted to the left in Fig. 3. C shows, from another experiment, a more rostral track along which units were driven by somatosensory stimuli as plotted to the right in Fig. 3, except for the most ventral unit (C11), which responded to auditory stimulation. Cats were prepared for single-unit recording by standard methods¹⁸ and were maintained under Flaxedil paralysis on N₂O anaesthesia supplemented with barbiturates. The microelectrode was advanced in 100- μ m steps and successive recording sites were numbered sequentially beginning at the top of the claustrum. Electrolytic marking lesions (10 μ m, 10 s, tip negative) were made at A15, B7, C1 and C13. The tracks of A, B and C were placed, respectively, 0.4, 2.4 and 6.0 mm in front of the caudal tip of the claustrum. Scale bar, 1 mm. D, Photomicrograph illustrating intermingled cell populations labelled in the claustrum by HRP injected into the ipsilateral area centralis representation of areas 17 and 18 and NY injected into the homotopic locus in the contralateral hemisphere. From a 50- μ m frontal section 1.1 mm ahead of the caudal tip of the claustrum and viewed under 360 nm illumination; dark cytoplasmic reaction product indicates HRP labelling and bright nuclear fluorescence indicates NY labelling. Scale bar, 200 μ m. The most dorsal HRP-positive neurones lie 300 μ m below the dorsal border of the claustrum. E, Dark-field photomicrograph of a transverse section through the somatosensory claustrum shows the results of a combined electrophysiological-neuroanatomical experiment. As the electrode was lowered along the track marked by the vertical white line, cells with receptive fields containing the hindleg were encountered first (E1–E3), followed by cells with receptive fields restricted to more rostral body parts (E4–E7). Subsequently, HRP was injected under electrophysiological guidance into the area of primary somatosensory cortex (SI) representing the hindleg. The dorsal zone of dust-like reaction product, indicative of HRP transported along corticofugal axons, overlaps closely the stretch of the track (E1–E3) along which hindleg responses were recorded. The track was placed 5.9 mm rostral to the caudal tip of the claustrum and a marking lesion was made at the bottom of the track (E7) (note erythrocytes at the site of the lesion labelled because of their endogenous peroxidase activity). Scale bar, 0.5 mm.



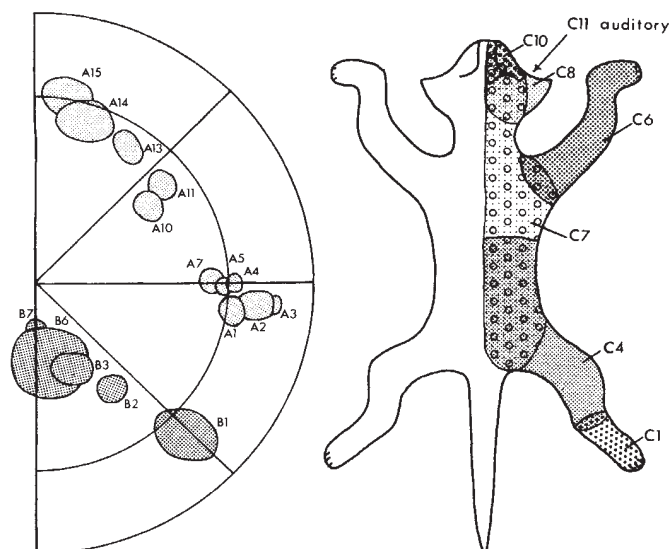


Fig. 3 Visual and somatosensory receptive fields of selected units recorded along the tracks shown in A, B and C of Fig. 2. The electrode was advanced in 100- μ m steps and recording sites were numbered consecutively in downward succession from the claustral surface. Visual receptive fields were plotted on a hemispheric screen, here shown in frontal projection with concentric circles representing 45° and 90° isodeviation lines. The pronounced upward progression of fields plotted during track A confirms anatomical evidence (not shown here) indicating that the extreme upper visual periphery is represented ventrocaudally in the claustrum, as in the cortex. The figurine map to the right illustrates the systematic caudorostral progression of receptive fields observed as units recorded at successively deeper sites along track C in the somatosensory claustrum. For clarity, only selected representative somatic fields are shown here. The unit at C11, recorded just below the region of somatic responsiveness, was excited by clicks or tone bursts delivered to either ear.

tree shrew area 17, the claustral projection arises in neurones of layer VI whereas the return pathway terminates in a number of layers including I and IV⁵. We have confirmed and extended these results in the cat, first, by injecting anterograde and retrograde tracers into the caudal claustrum and observing patterns of transported label in area 17, and, second, by comparing the cell populations in area 17 projecting, respectively, to the claustrum and to the lateral geniculate body (LGD). The anterograde results suggest that in the cat the claustrum projects densely to layer I, but there was also heavy labelling of deeper layers, including IV. The retrograde results indicate that the projection to the claustrum arises from cells in layer VI. These cells seem to be distinct from those projecting to the LGd¹⁴, for in two cases intermingled but separate layer-VI populations were labelled following placement of distinguishable retrograde tracers (NY and HRP) in retinotopically matched parts of the claustrum and the LGd.

The claustrum bears a unique resemblance to the thalamus by virtue of its extensive reciprocal connections with the neocortex. We have extended this parallel by showing that the claustrum, like the thalamus, contains discrete subdivisions that are tightly interlinked with sensory cortex and are organized topographically. However, in lacking major ascending sensory connections, the claustrum fails to share at least the relay functions of the thalamus and seems, in its sensory afference, to be a satellite of the neocortex. This is a rare pattern, a second instance being the almost exclusive dependence of the parabigeminal nucleus on the superior colliculus¹⁵. Like the sensory divisions of the claustrum, the parabigeminal nucleus is a small cell group receiving a topographic projection from a subset of cells in a much larger structure to which it returns a topographically organized projection^{15,16}. Why either cell group should exist as a satellite and not as a set of interneurons within the parent structure is an intriguing question to which no satisfactory answer has been obtained.

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Note added in proof: Since submission of this paper, the existence of retinotopic organization in the claustrum has been reported independently¹⁹.

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Dorsal column input into the reticular formation

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Somatic sensory information, such as touch, pressure, pain and body position, is conveyed to the mammalian thalamus and ultimately the cerebral cortex along three major ascending pathways in the spinal cord: (1) the dorsal column medial lemniscal (DCML) system, (2) the ventral column (VC) system, including the 'neospinothalamic', 'paleospinothalamic' and spinothalamic tracts, and (3) the spino-cervico-lemniscal (SCL) system^{1,2}. The phylogenetically recent DCML system has been traditionally viewed as an exclusive and homogeneous one-to-one relay of somatic sensory information to the thalamus³. The relation of the ascending spinal pathways to the brain stem reticular formation (RF) is of particular interest in view of the numerous important functions subserved by this region. The spinal input into the RF is generally considered to reach it exclusively through the VC⁴. In view of their intraspinal connections⁵⁻⁸, it is difficult to separate and interact the three ascending spinal pathways. We now demonstrate a DCML input into the gigantocellular nucleus (Rgc) of the medial medullary RF in cats, and show inhibitory as well as excitatory interactions between DCML and VC tracts in RF cells. We used a combination of careful spinal surgical lesions aimed at disconnecting the DCML from the other afferent systems and of localized spinal and peripheral stimulations. Persistence of the response of Rgc neurones to DCML stimulation in decerebrate and decerebellate cats rules out the possibility of descending activation via a feedback loop from the cerebral and cerebellar cortices. In view of the possible involvement of Rgc in processing nociceptive input, these findings are of interest to our understanding of the DCML system in somatosensory function and, in particular, pain mechanisms.

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Experiments were carried out on 17 adult cats, anaesthetized with 30 mg per kg pentobarbitone sodium (9 cats) or 70 mg per kg chloralose (3 cats), or decerebrated just rostral to the superior colliculus following induction of anaesthesia with ether (5 cats). All animals were subsequently paralysed with gallamine triethiodide and artificially respired. Body temperature, blood pressure and end-tidal CO_2 were maintained within physiological levels. Laminectomy was performed from C_1 to C_5 followed by suboccipital craniectomy and bilateral exposure of the sensorimotor cortex. A coaxial electrode was placed stereotactically in the medial lemniscus at coordinates A 5, L 4.5, V 1.8.

In all cats, the cerebellar connections to the brain stem were interrupted and one of two types of spinal lesion was performed. In the first, the 'dorsal-cut' (d-cut) preparation, the dorsal half of the spinal cord was completely severed at the level of C_1 or C_3 (in the latter case, the upper three cervical dorsal roots were also cut) or at both levels. The completeness of the cut was tested electrophysiologically. This preparation eliminates DCML and SCL inputs but allows VC inputs to reach RF following peripheral cutaneous stimuli. In the second, the 'ventral-cut' (v-cut) preparation, the entire spinal cord, except for dorsal columns on both sides, was severed at C_1 or C_3 . This preparation leaves the dorsal columns (DC) as the sole connection between the periphery and the brain. At the end of each experiment, the cat was perfused with a 10% formaldehyde solution through the aorta. The whole brain (in non-decerebrate cats) and spinal cord were removed and Nissl-stained. All the lesions were later verified for completeness and accuracy. Experiments where histological lesions were imperfect were discarded.

Electrical stimuli were delivered at the rate 0.5–1 Hz to the DC or ventrolateral tracts of VC through stainless steel bipolar electrodes with 1-mm tip separation and to the central footpad of each limb through needle electrodes. Stimuli consisted of single pulses of 0.05–0.1 ms duration. Occasionally, a train of three pulses was delivered to the VC or the paws. The current pulse strength (peak intensities of 0.5–2.5 mA) and the special precautions to insure that the spinal cord stimuli were localized to the stimulated tracts are described elsewhere⁹. Peripheral stimuli would be expected to be carried into the brain along VC tracts in d-cut cats and along DC tracts in v-cut cats.

We studied 160 Rgc units through microelectrodes at coordinates P 3 to 7, L 1.5 and V –7 to –11. Microelectrode recording sites were marked by the electrocoagulation technique. Most (154) of the units were initially isolated after a DC searching stimulus and 6 units after VC stimulation. DCML-activated cells were widely distributed in Rgc and their occurrence correlated

well with the amplitude of the gross potential evoked by DC stimulation. These cells responded to contralateral DC (CDC), ipsilateral medial lemniscal and, most interestingly, to peripheral stimulation in v-cut cats (Fig. 1). The CDC activation pattern consisted of one or more spikes per discharge. Spontaneous activity, when present, was also modified. CDC stimulation (just above C_1 cut) evoked a response which ranged in latency between 2 and 14 ms. The shorter latency units showed a frequency following up to 50 Hz (but usually less than 25 Hz). Medial lemniscal stimulation was tested in 91 units in non-decerebrate cats, 66 of which responded, usually with latencies and frequency following characteristics similar to those evoked by CDC stimulation.

Of 82 units studied in v-cut cats, most (79 units) responded to DC stimulation and 53 responded to electrical stimulation of the footpads (Fig. 1c, d). The natural receptive fields could be determined in 20 of these cells (18 responded to touch, 2 to hair stimulation). Such fields were usually well defined and circumscribed.

Of the total 160 units studied, 110 were activated through the VC following stimulation of either the VC directly along the ventrolateral tracts or the peripheral paws in d-cut cats. In the latter, unit responses evoked by direct VC stimulation were similar to those evoked by peripheral stimulation, except for slightly shorter latencies. These units were activated by electrical stimuli to all four paws and, in some instances, by acoustic stimuli as well. Some of these units were stimulated or inhibited by noxious pinch at several peripheral sites. Thus, compared with units responding to CDC stimulation in v-cut cats, these units showed more diffuse receptive fields and somewhat longer initial spike latencies.

Interactions between DC and VC inputs on Rgc neurones were studied using conditioning-testing techniques in 79 units discharging to both DC and VC inputs and 32 units discharging to one input only. All (except two) units demonstrated an interaction between the two inputs which took the form of inhibition (48 units) or initial facilitation for 30–40 ms followed by inhibition (61 units). Inhibition lasted up to 200–600 ms.

These results strongly support the existence of an input from the DCML system into the RF which also interacts with the well known VC inputs. We cannot explain the failure of recent physiological studies^{10–12} to observe these connections. The neuroanatomical substrate for this DC–RF route also remains unclear except perhaps for the brief description of sparse projections from the DC nuclei into the RF with silver degeneration stains^{13–15} or in Golgi-stained serial sections¹⁶. Similar sparse projections have been described from the dorsal roots (presumably through the DC) to the RF¹⁷. It is difficult to ascertain whether the dense DC nuclear projection to the inferior olive¹⁸, which in turn connects reciprocally to the RF¹⁹, could mediate the DC influences on the RF described here. However, RF projections into DC nuclei have recently been described^{20–23}.

The above DC–RF connection could be part of a 'palaeolemniscal' subsystem of the DCML system recently shown to originate from distinct parts of the DC nuclei, to possess a significant number of post-primary DC fibres, and to be characterized by a more diffuse arrangement of neurones with widespread efferents into thalamic (other than VPL) and brain stem areas²⁴. The DC–Rgc route and the DC–VC interactions on Rgc neurones described here support the hypothesis, reviewed by Dennis and Melzack²⁵, implicating the palaeolemniscal component of the DCML system in pain mechanisms.

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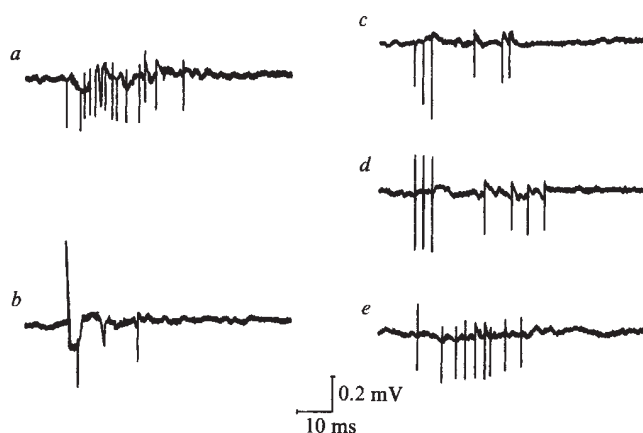


Fig. 1 Sample records showing the responses of an Rgc neurone in a v-cut preparation to electrical stimulation of the following sites. *a*, Contralateral dorsal column; *b*, ipsilateral medial lemniscus; *c*, contralateral forepaw; *d*, ipsilateral forepaw; *e*, ipsilateral ventrolateral column. Single pulses were used in *a*, *b* and *e*, and a train of three pulses at 300 Hz was used in *c* and *d*.

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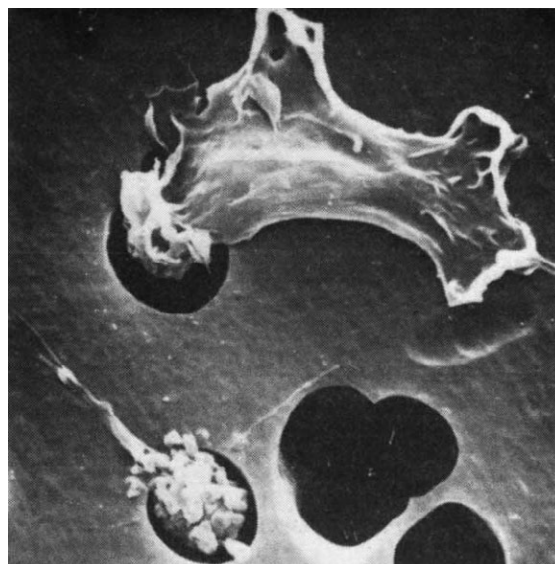


Fig. 1 Scanning electron micrograph of the lower surface of a porous membrane, showing two successive stages of endothelial cell migration through the membrane. The cell shown in the lower half of the field has extended only a slender cell process to attach to the undersurface of the membrane. The cell in the upper half of the field has extended a large pseudopod through a pore to attach to the undersurface of the membrane. $\times 4,900$.

Adult tissues contain chemo-attractants for vascular endothelial cells

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New blood vessel formation, or angiogenesis, occurs through the migration of endothelial cells in elongated sprouts. These sprouts are directed preferentially towards the inciting stimulus^{1–3}. Several studies have demonstrated that certain chemical substances can stimulate angiogenesis^{4–7}. In these cases, endothelial cell migration towards the chemical stimulus may be due to a preferential migration of cells from lower to higher concentrations of the mediator. Such concentration gradient-dependent cellular migration has been termed chemotaxis⁸. Using a modification of the Boyden chamber technique⁹ to measure chemotaxis *in vitro*, we have now found that extracts of various adult bovine tissues have potent chemotactic activity for vascular endothelial cells. Adult bovine serum lacks similar chemotactic activity.

The blind well Boyden chamber⁹, commonly used for studying chemotaxis, consists of two wells (an upper well and a lower well) separated by a porous membrane. A known concentration of the chemoattractant to be tested is placed in the lower well. The upper well receives a predetermined number of cells and, in some experiments, a known concentration of chemoattractant. The cells we used were fetal bovine aortic endothelial cells, prepared as described previously^{7,10}. The cells attach to the upper surface of the membrane, migrate through the pores and then attach to the lower surface of the membrane (Fig. 1). We have found that, unlike some other cell types^{11,12}, less than 5% of the vascular endothelial cells detach from the lower surface of the membrane during the experiment. Therefore, the number of cells found on the lower surface of the membrane accurately reflects the number of endothelial cells which have migrated through the pores. The relationship between the number of cells migrating through the porous membrane and the concentration gradient of attractant across the membrane can then be

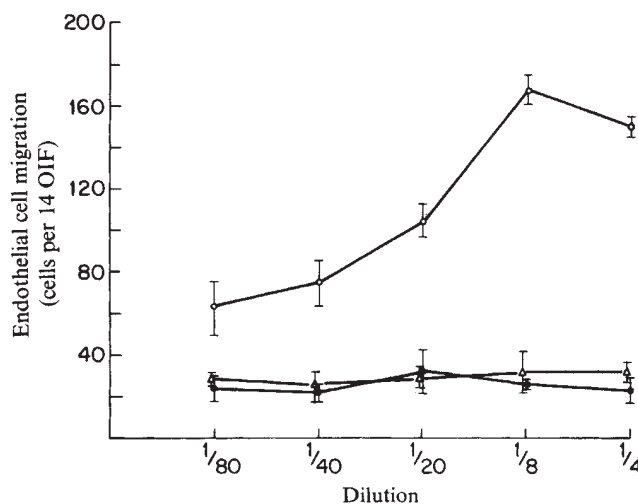


Fig. 2 Effect of bovine retinal extract on vascular endothelial cell migration. Migration is measured using blind well chambers (Neuroprobe, no. 200-312) and polyvinylpyrrolidone-free polycarbonate membranes with 5- μ m pores (Nucleopore). Retinal extract is prepared by incubating bovine retinas in BSS at room temperature for 3 h (ref. 7). The suspension is centrifuged and the supernatant used immediately or stored at -20°C (ref. 7). The lower wells receive retinal extract (○), BSS (●) or ABS (△) (Sterile Systems) diluted in minimal essential medium with 10% fetal bovine serum (MEM/10). The upper wells receive 175,000 fetal bovine aortic endothelial cells in 0.8 ml MEM/10 without added retinal extract, salt solution or adult serum. The migration chambers are then incubated at 37°C in 5% CO_2 with 85% relative humidity for 7 h. The cells on the upper surface of the membranes are then removed with a cotton swab. The cells which have migrated through the membrane on to the lower surface are fixed in a 10% neutral buffered formalin and stained with Wright's stain. Migration is quantified by counting the number of cell nuclei on the lower surface in 14 oil immersion fields (OIF). Each point represents the mean of three experiments $\pm$ s.e.m. To measure cell attachment to the membranes, each membrane is rinsed in BSS, treated with 0.3 ml of 0.1% trypsin for 5 min, and the trypsin activity quenched by the addition of 0.7 ml MEM/10. The cell number and Trypan blue exclusion are then determined using a haemocytometer. Attachment and viability are measured at hourly intervals during the experiment.

Table 1 Vascular endothelial cell chemotaxis induced by retinal extract

% Retinal extract below membrane	0	% Retinal extract above membrane	2.5	5	10
0	46 (9)	71 (9)	56 (4)	77 (10)	
2.5	107 (13)	90 (6)	89 (5)	106 (15)	
5	175 (12)	157 (5)	124 (3)	140 (10)	
10	214 (18)	208 (13)	183 (13)	142 (13)	

The chemotactic activity of retinal extract was determined using the migration assay as described in Fig. 2 legend, except that various amounts of retinal extract are added to both upper and lower wells to produce a series of concentration gradients across the membranes. Migration is quantified by counting the number of cell nuclei on the lower surface of the membrane in 14 oil immersion fields. Each number represents the mean of three experiments ($\pm$ s.e.m.). The values in the diagonal, where there is no gradient, show the chemokinetic effects of retinal extract.

determined, thus providing a measure of the chemotactic activity of that substance for vascular endothelial cells *in vitro*.

To investigate the stability of the concentration gradients across the membranes, various amounts of the tricarboyanine dye indocyanine green (ICG) (maximum final concentration of $0.5 \mu\text{g ml}^{-1}$) were added to the upper and lower wells of the migration chambers. ICG binds rapidly to serum proteins, mainly albumin (95%)¹³. The migration assay was then carried out as described in Fig. 2 legend. The concentration of ICG in the upper and lower wells was determined at various times by spectrophotometry. After 4 and 7 h the concentrations varied from their initial values by less than 10% and 20%, respectively. Although we cannot determine the concentration gradient of chemoattractant in the immediate area of the porous membrane, the relative stability of the concentration gradients of ICG-labelled proteins suggests that the initial gradient is a good estimate of the gradient that affects the cells.

Extracts of adult retinas from several mammalian species have been found to be potent stimulators of angiogenesis⁷. We therefore studied their effect on vascular endothelial cell migration to establish that vascular cells could be stimulated to migrate through the porous membranes. The addition of adult bovine retinal extract to the lower well of the migration apparatus results in a marked, dose-dependent increase in the number of endothelial cells moving through the porous membrane as compared with control wells to which balanced salt solution (BSS) is added (Fig. 2). In contrast, adult bovine serum (ABS) does not stimulate endothelial cell migration. The presence of retinal extract, BSS or ABS in the concentrations tested does not affect the rate of endothelial cell attachment or the absolute number of viable cells attaching to the porous membranes (Fig. 2 legend).

These results demonstrate that retinal extract is a potent stimulator of vascular endothelial cell migration but do not indicate whether the effects of the extract are chemotactic or chemokinetic (that is, due to chemically stimulated migration not dependent on concentration gradients). To investigate this,

Table 2 Vascular endothelial cell chemotaxis induced by various tissue extracts

% Extract below membrane	0	% Extract above membrane	2.5	5	10
0	46 (10)	73 (2)	91 (12)	119 (7)	
2.5	159 (17)	154 (3)	158 (3)	139 (6)	
5	226 (16)	191 (5)	153 (3)	139 (5)	
10	256 (12)	215 (2)	178 (12)	149 (8)	

The chemotactic activity of extracts of adult bovine liver, kidney, heart and skeletal muscle was determined as described in Table 1 legend. These extracts were prepared in the same manner as retinal extract (Fig. 2). The chemotactic activity of liver extract is shown above; extracts of the other tissues have similar activity.

we varied the concentration gradient of retinal extract across the membranes and noted the effect on endothelial cell migration. The results shown in Table 1 demonstrate that vascular endothelial cells *in vitro* migrate preferentially from a lower to a higher concentration of bovine retinal extract, and that the extract thus has a chemotactic effect on these cells.

Recent studies on the ability of other adult tissues to stimulate and support new blood vessel growth^{7,14-16} have occasionally produced conflicting results. We prepared extracts of adult bovine liver, kidney, heart and skeletal muscle as previously described⁷ and found that all have chemotactic activity for vascular endothelial cells *in vitro* (Table 2). These extracts have no effect on the adherence of endothelial cells to the membranes or on endothelial cell viability. The chemotactic activities of all tissue extracts tested are nondialysable (molecular weight cut-off 10,000–12,000), are not retained by an Amicon XM-100 filter (molecular weight cut-off 100,000) and are inactivated by boiling for 2 min.

As vascular endothelial cell migration and chemotaxis seem to be essential for angiogenesis, it is important to study these phenomena in a quantitative *in vitro* system where various parameters can be readily controlled. We have developed a simple, rapid (6–8 h), reproducible and quantitative assay of vascular endothelial cell chemotaxis *in vitro*, and have used it to demonstrate significant chemotactic activity in extracts of various adult bovine tissues. Further study of tissue-derived chemotactic activity should improve our understanding of neo-vascularization. Furthermore, it is reasonable to assume that chemotactic behaviour in endothelial cells has a crucial role in developmental events.

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Leukotrienes are potent constrictors of human bronchi

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Slow reacting substance of anaphylaxis (SRS-A) is released by various stimuli, including immunological challenge, and has long been considered an important mediator of immediate hypersensitivity reactions, such as bronchoconstriction in allergic asthma¹⁻³. Recently, slow reacting substances from several tissues have been identified and characterized as members of a newly discovered group of substances, the leukotrienes⁴. Leukotrienes are generated from arachidonic acid and other polyunsaturated fatty acids in a pathway initially involving a lipooxygenase-catalysed oxygenation at C-5 (Fig. 1). This differs

from the synthesis of prostaglandins and thromboxanes, where the initial transformation of arachidonic acid is catalysed by a cyclo oxygenase (Fig. 1). Recently, leukotriene C_4 (LTC_4 : 5(*S*)-hydroxy, 6(*R*)-*S*-glutathionyl-7,9-*trans*, 11,14-*cis*-eicosatetraenoic acid) and D_4 (LTD_4 : 5(*S*)-hydroxy, 6(*R*)-*S*-cysteinylglycyl-7,9-*trans*, 11,14-*cis*-eicosatetraenoic acid) were found to have biological effects in several bioassay systems, which are strikingly similar to those previously reported for impure extracts of SRS-A^{5,6}. Here we report the remarkable contractile activity of both LTC_4 and LTD_4 on isolated human bronchi, which further emphasizes the possibility that leukotrienes are potent mediators of bronchoconstriction in man.

Macroscopically normal parts were selected from human lung parenchyma excised because of carcinoma, and immediately after removal placed in ice-cold Tyrode solution. Bronchi with a diameter of 2–4 mm were cut into helical strips and suspended in a 5-ml organ bath filled with Tyrode solution containing (mM): NaCl 149, KCl 2.7, $NaHCO_3$ 11.9, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.4 and glucose 5.5, kept at 37°C gassed with 5% CO_2 in O_2 . Contractions were recorded on a Grass Model 7 Polygraph using an isometric Grass FT 03C force displacement transducer (resting tension 5 mN). Drug concentrations are expressed as final concentration in bath fluid surrounding the tissue. Leukotrienes C_4 and D_4 were generated biosynthetically, as described previously^{7–9}. LTA_4 was obtained by hydrolysis of its methyl ester, and was dissolved in ethanol whereas LTC_4 and LTD_4 were dissolved in 0.9% NaCl:ethanol (5:1). Care was taken to avoid ethanol concentrations in the bath above 0.05%, because ethanol can itself act on the preparation.

LTC_4 and LTD_4 , as well as histamine, caused dose-dependent and long-lasting contractions of human bronchial strips (Fig. 2). The contraction induced by histamine readily disappeared after changing of the bath fluid, whereas the response to both LTC_4 or LTD_4 vanished only very slowly even after repeated washing. In fact, a contraction obtained by a high dose of LTC_4 (100 nM), although reversible by isoprenaline (1 μ M), reappeared when added drugs were washed out.

The proposed SRS-A antagonist FPL 55 712 (1–10 μ M) reversed the contraction induced by LTC_4 or LTD_4 , whereas the

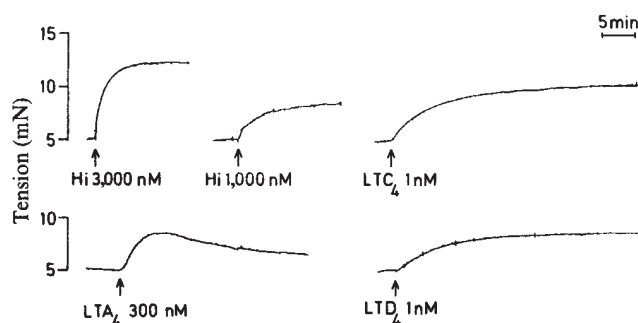


Fig. 2 Contraction responses to histamine (Hi), LTC_4 , LTD_4 and LTA_4 in isolated human bronchi. Drugs added at arrows, wash indicated by broken curve.

histamine response was largely unaffected (not shown). Blockade of muscarinic and histamine-1 receptors by atropine (1 μ M) and mepyramine (1 μ M), respectively, had no effect on the LTC_4 -contraction.

Because repeated administration of leukotrienes seemed to cause tachyphylaxis, we used non-cumulative administration of drugs to assess their potency. The log dose-response curves thus obtained for histamine and LTC_4 almost paralleled and had similar maximal values, but the LTC_4 curve was markedly to the left of histamine. The regression lines calculated from the dose-response relationships of the two compounds showed a 100% increase in resting tension of the preparation ($ED_{100\%}$) obtained with 6.3 nM LTC_4 and 10 μ M histamine (Fig. 3). This indicates that, on a molar basis, LTC_4 is at least 1,000 times more potent than histamine in causing contraction of isolated human bronchi.

In some experiments, the contractile effect of LTC_4 was compared with that of LTD_4 (Fig. 2). There were no large differences in potency between the two compounds in either the low or high concentration range. However, $PGF_{2\alpha}$, recognized as an effective bronchoconstrictor¹⁰, was always more than 500 times less potent than LTC_4 . The close similarity between LTC_4 and LTD_4 in causing contraction of the human bronchi agrees well with their equipotency as bronchoconstrictors in the guinea pig lung both *in vitro* and *in vivo*⁵, although it has been suggested that LTD_4 is more potent than LTC_4 in the guinea pig parenchymal strip¹¹. Whether the observed similarity in potency between LTC_4 and LTD_4 is due to a rapid conversion of administered LTC_4 into LTD_4 by the enzyme γ -glutamyl transpeptidase (GGTP)^{8,12}, tissue catabolism of LTD_4 , or a true equipotency as contractile agents on bronchial smooth muscle, remains to be established.

LTA_4 , the unstable epoxide intermediate formed from 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE)⁴ (Fig. 1), was less potent than both LTC_4 and LTD_4 , but almost as potent as histamine in causing contraction of the bronchial strip (Fig. 2). The effect of LTA_4 seemed to be more short-lived than that of LTC_4 or LTD_4 and sometimes the contraction faded before washing. This could be due to spontaneous breakdown of LTA_4 in the organ bath or enzymatic transformation of the substance in the tissue.

It has been shown that SRS-A consists of a mixture of leukotrienes^{11,13}, with LTC_4 as the primary product, and depending on the type of tissue and experimental conditions, varying amounts of LTD_4 , and other leukotriene metabolites are formed^{12,14}. It has not been established whether LTC_4 or LTD_4 is predominantly formed in human lung tissue *in vivo* but both compounds are highly potent bronchoconstrictors. They both increase the vascular permeability in guinea pig skin⁵; thus leukotrienes must seriously be considered as possible pathological agents causing both the bronchospasm and mucosal oedema of bronchial asthma.

The finding that arachidonic acid can be transformed into leukotrienes might explain the failure of aspirin, and other non-steroidal anti-inflammatory drugs, to alleviate allergic

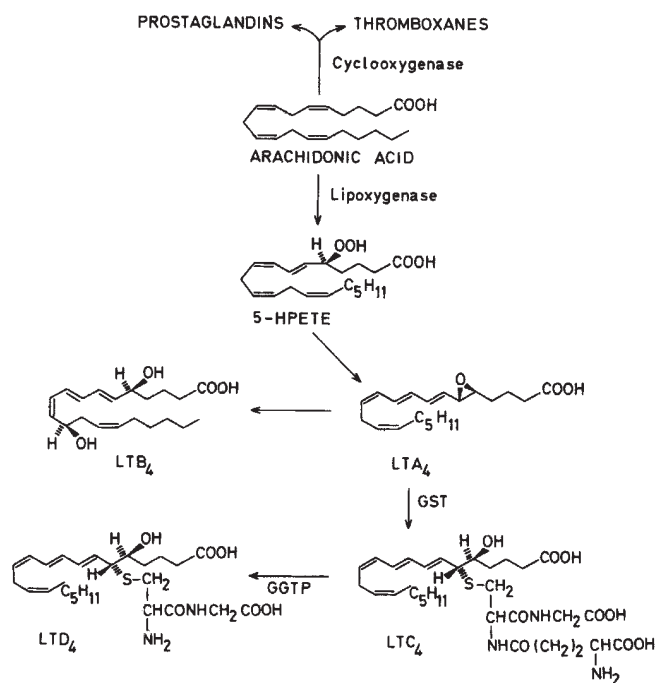


Fig. 1 Conversions of arachidonic acid into oxygenated derivatives. LTA_4 : leukotriene A_4 , etc. (subscript denotes number of double bonds)¹⁷. 5-HPETE: 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid. GST: glutathione *S*-transferase. GGTP: γ -glutamyl transpeptidase.

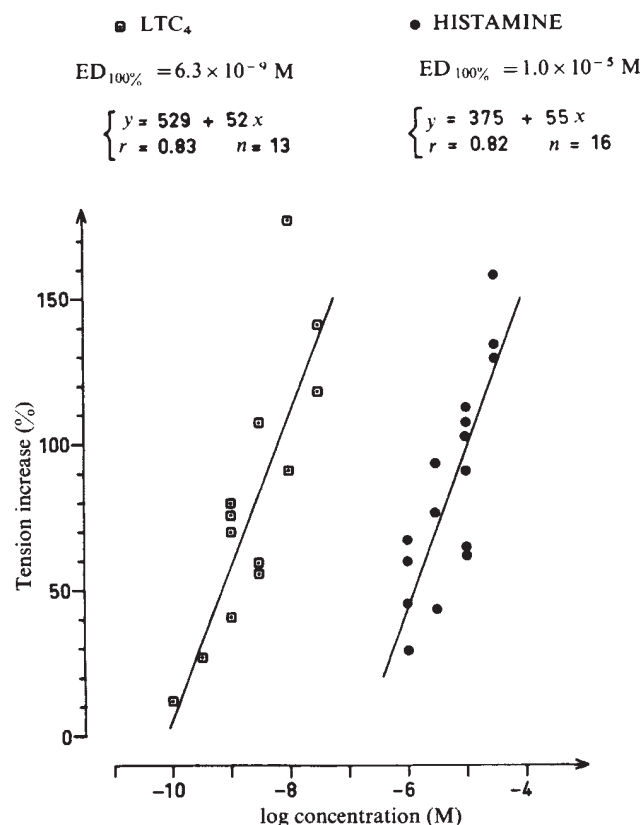


Fig. 3 Contraction responses to histamine (●, $n = 16$) and LTC₄ (□, $n = 13$) plotted on a semilogarithmic scale. Regression lines calculated according to the method of least squares. ED_{100%}, dose of agonist increasing tension 100% above resting level (5 mN). Results for 13 strips obtained from eight subjects.

bronchoconstriction, because these drugs block the cyclooxygenase, but not the lipoxygenase¹⁵ (Fig. 1). It is even possible that in 'aspirin-sensitive' asthma, inhibition of the cyclooxygenase by aspirin could contribute to the symptoms by diverting arachidonic acid metabolism into formation of the potent leukotriene bronchoconstrictors, while formation of prostaglandins inhibiting mediator release¹⁶ is decreased. In addition, the ability of LTC₄ and LTD₄ to cause release of the potent bronchoconstrictor and cyclooxygenase product, thromboxane A₂ (Folco *et al.*, personal communication), further illustrates the complex situation concerning the formation of arachidonic acid-derived products with biological effects relevant to bronchial asthma. The interrelationship between prostaglandins, thromboxanes and leukotrienes in immediate hypersensitivity reactions obviously requires further study. The results presented here demonstrate that the leukotrienes C₄ and D₄ could be of considerable importance in the pathophysiology of human asthma.

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Monoclonal antibodies to lymphocytic choriomeningitis virus react with pathogenic arenaviruses

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Certain arenaviruses have become widely recognized as important human pathogens^{1–3}, the most notable among these being Lassa virus, the causative agent of Lassa fever⁴. Two other members of the group, Junin and Machupo virus, are the aetiological agents of Argentine and Bolivian haemorrhagic fevers, respectively². All these agents share both morphological features and to varying degrees serological cross-reactivity with other non-pathogenic arenaviruses^{3,5,6}. Despite the evident clinical importance of these viruses, work to define their physicochemical structure and to develop rapid and precise diagnostic techniques has been slow. Consequently, the definitive relationships among West African Lassa strains, strains of a related agent from Mozambique and of an Old World arenavirus, lymphocytic choriomeningitis (LCM), have not been established. This problem is of more than simple taxonomic importance in view of the fact that a Mozambique virus strain produced subclinical infection in experimental monkeys which were then resistant to challenge with monkey and human virulent Lassa virus from Sierra Leone⁷. We have explored the use of monoclonal hybridoma antibodies generated against relatively less hazardous arenaviruses to define antigens cross-reactive with the important human pathogens of the group. Here we describe the use of monoclonal antibodies directed against LCM virus to define antigenic specificities shared among LCM, Lassa and Mozambique viruses.

Hybridoma cell lines were generated by fusion of spleen cells from immunized mice with P3 × 63 Ag8 mouse myeloma cells, and selected in hypoxanthine-aminopterin-thymidine (HAT) medium as described elsewhere^{8,9}. Immunization of spleen cell donor mice was accomplished by intraperitoneal (i.p.) inoculation of BALB/c mice with 10⁴ plaque-forming units of LCM virus (Armstrong Strain, CA 1371) followed by a second inoculation of 3–7 µg of purified LCM virus given intravenously 3 days before fusion. The spleen cells obtained were fused at a ratio of 10 spleen cells per myeloma cell using polyethylene glycol 1000. Cells were placed in microtitre wells in HAT medium, and wells showing growth were screened for antibody to LCM virus by indirect immunofluorescence¹⁰. Wells showing evidence of specific antibody were cloned by limiting dilution and the resultant clones again screened for specific antibody either by indirect immunofluorescence or by solid-phase radioimmune assay. Final determination of specificity of the cloned antibodies was made by radioimmune precipitation assay

Table 1 Cross-reactivity of LCMV hybridoma antibodies with Lassa and Mozambique viruses

Hybridoma antibody	Immunoglobulin isotype	LCMV polypeptide specificity	Indirect immunofluorescence titre ($\times 10^{-1}$) against:		
			LCMV	Lassa	Mozambique
1-1-3 (MAF)	IgG2A	NP	12,800	200	3,200
24A-21 (MAF)	IgG2A	NP	51,200	51,200	51,200
25-39 (MAF)	IgG2A	NP	12,800	6,400	6,400
24 B-2 (TCF)	IgG1	NP	100	100	100
3-3 (TCF)	ND	ND	8	8	8
9-7-5 (MAF)	IgG2A	GP-2	800	<2	400

MAF: mouse ascites fluid; TCF: tissue culture fluid; ND: not done; NP: 63,000 molecular weight nucleocapsid protein of LCM virus; GP-2: 35,000 molecular weight glycoprotein of LCM virus¹¹.

as described elsewhere¹¹. Ascites fluids were obtained by inoculation of 5×10^6 to 10^7 hybridoma cells i.p. into pristane-primed BALB/c mice.

Cross-reactivity was assessed by indirect immunofluorescent staining of spot slides of cells infected with Lassa or Mozambique viruses¹². These slides were prepared in the maximum containment facility at CDC, Atlanta, and were fixed in acetone. Fluorescein isothiocyanate-conjugated goat antibody to mouse IgG was prepared in this laboratory. Infected cells stained with serial twofold dilutions of culture fluid or ascites from individual hybridoma cultures were examined using a Zeiss fluorescence microscope equipped with a 100-W mercury lamp and epilluminator. The results reported are the highest dilution giving positive staining. Controls included LCM virus-infected cell preparations (positive control) as well as uninfected cells. Control hybridoma culture fluids directed against unrelated viral (Pichinde virus, measles) and non-viral antigens (P3 $\times$ 63 Ag8 culture fluid) were used.

Forty-six monoclonal antibody-producing hybridoma cell lines with specificity for LCM virus were analysed for cross-reactivity with Lassa and Mozambique viruses. Of these, 9 reacted against virus-specific determinants expressed at the surface of the LCM-infected cell (M. J. B., unpublished observations). Of the remaining 37 antibodies, most recognized determinants on the viral nucleocapsid protein, although some remain uncharacterized.

To assess the fine specificity of these monoclonal antibodies, we reacted each against two strains of LCM virus differing in their pathogenicity for guinea pigs. These viruses, LCMV Armstrong (ARM)¹³ strain and LCMV-WE¹⁴ strain, have not been previously observed to differ serologically. When our panel of nine monoclonal antibodies which reacted at the surface of infected cells was tested against target cells infected with either virus, six antibodies were found to be cross-reactive, two defined determinants unique to LCMV-ARM and one reacted only with LCMV-WE. These results are also of interest because of the low pathogenicity of LCMV-ARM in humans when compared with LCMV-WE. Thus, differences in the virus-specific surface antigens, previously unknown using conventional reagents, were resolved. Similar strain-specific antigenic determinants were not observed in the nucleocapsid protein using our panel of reagents, presumably indicating conservation of this viral polypeptide.

Lassa virus had previously been shown to cross-react with LCM virus by complement fixation^{1,7,15} and indirect immunofluorescence¹⁶ tests. We therefore assayed target cells infected with Lassa and Mozambique viruses with our panel of monoclonal antibodies directed against LCM virus, to identify cross-reactive determinants. A panel of 46 monoclonal antibodies was screened. The results summarized in Table 1 showed that five cross-reacted with both viruses and a sixth reacted only with Mozambique virus. Cross-reactivity with Lassa virus was limited to antibodies which recognize determinants on the nucleocapsid protein of LCM virus.

Such antibodies, notably 1-1-3 and 24A-21 (Table 1), did not react at 1:10 dilution to any of the antigens prepared with all

eight New World arenaviruses of the Tacaribe antigenic complex. This pattern suggests that Old World and New World arenaviruses have evolved independently over a long period. These antibodies also provide a convenient method for initial screening of candidate Old World arenaviruses.

Of particular interest was antibody 9-7-5, which reacted with the Mozambique agent but not West African Lassa virus. This antibody, which recognizes the GP-2 glycoprotein on LCM virus-infected cells (M.J.B., unpublished observations), thus enables Mozambique strains to be specifically segregated by serological test. In further tests, we found that antibody 9-7-5 reacted with each of nine 'Mozambique' strains recovered from *Mastomys natalensis* rodents in that country and in Zimbabwe, whereas it failed to detect antigens produced by six West African Lassa strains of human and rodent origin obtained from Nigeria, Liberia and Sierra Leone.

Monoclonal antibodies have proved to be useful tools in analysing virus-specific antigens. The present study demonstrates that antibodies raised *in vitro* against LCM virus, the prototype virus of the arenavirus group, can be used to analyse cross-reactive viruses in the group. This ability to raise useful cross-reactive monoclonal antibodies may make it possible to circumvent the need to grow dangerous pathogens such as Lassa virus yet still produce useful and precise clinical reagents. Such reagents would be expected to facilitate the rapid and accurate identification of virus isolates. In particular, the reaction of the anti-glycoprotein monoclonal antibody 9-7-5, which specifically cross-reacts with Mozambique virus but not Lassa virus, suggests that Mozambique virus shares homology in the envelope glycoprotein with LCM virus. We are now expanding our panel of monoclonal reagents both to facilitate the precise identification of clinical isolates and to analyse further the antigenic relatedness among arenaviruses.

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Production of human hybridomas secreting antibodies to measles virus

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Monoclonal antibodies against a variety of antigens can be produced using techniques of somatic cell hybridization between cells of rodent myeloma lines and B cells derived from animals immunized against a given antigen^{1,2}. However, because the monoclonal antibodies secreted by these hybridomas are of rodent origin, their use in human immunotherapy is limited. Thus the production of B-cell hybrids that secrete human monoclonal antibodies may be of considerable value. We have hybridized a hypoxanthine phosphoribosyl transferase (HPRT)-deficient human B-cell line derived from a patient suffering from multiple myeloma with peripheral lymphocytes obtained from a patient with subacute sclerosing panencephalitis (SSPE). These hybridomas were found to secrete human IgM specific for measles virus nucleocapsids.

As human chromosomes are lost in mouse × human hybridomas^{3,4} it is difficult to obtain interspecies hybridomas that secrete human antibodies, because such hybrids must retain both of the two human chromosomes that carry the rearranged genes for the human light and heavy immunoglobulin chains^{3,4}. The chromosomal constitution of intraspecific human hybrids is much more stable⁵; therefore we decided to use a HPRT-deficient human B-cell line derived from a patient with multiple myeloma to produce human × human hybridomas. We mutagenized human B-cell line GM 1500 (obtained from the Human Cell Depository, Camden, New Jersey)³ with ethyl-methane sulphonate (EMS) and selected the mutagenized cells in the presence of 30 µg ml⁻¹ 6-thioguanine. Two independent mutants, GM 1500 6TG-A1 1 and GM 1500 6TG-A1 2, were obtained. They did not express HPRT activity and died in

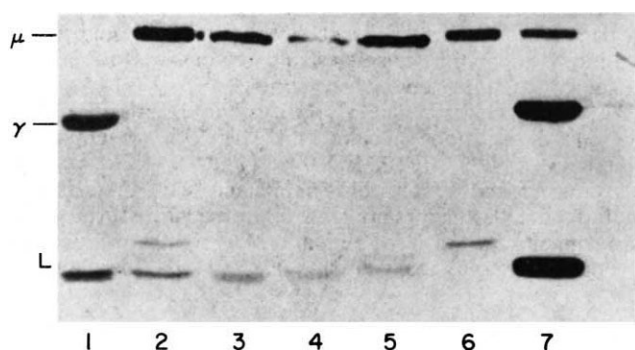


Fig. 1 Immunoprecipitation and 10% SDS-polyacrylamide gel electrophoresis of secreted human immunoglobulin chains produced by human hybridomas. Hybridoma cultures were labelled with 100 µCi ³H-leucine (70 Ci per mmol) per ml for 12 h. The human immunoglobulin chains were immunoprecipitated with rabbit anti-human heavy chain antigen using established procedures^{3,4}, then separated by 10% SDS-polyacrylamide gel electrophoresis as described elsewhere^{3,4}. Lane 1, immunoprecipitates of the immunoglobulin produced by GM 1500 6TG-A1 2 cells after reaction with an anti-human γ antiserum; lanes 2–6, immunoprecipitates of immunoglobulin chains secreted by human × human hybridomas after reaction with anti-human μ antiserum; lane 7, immunoprecipitate of immunoglobulin chains secreted by hybridoma D3 after reaction with anti-human μ and γ antiserum.

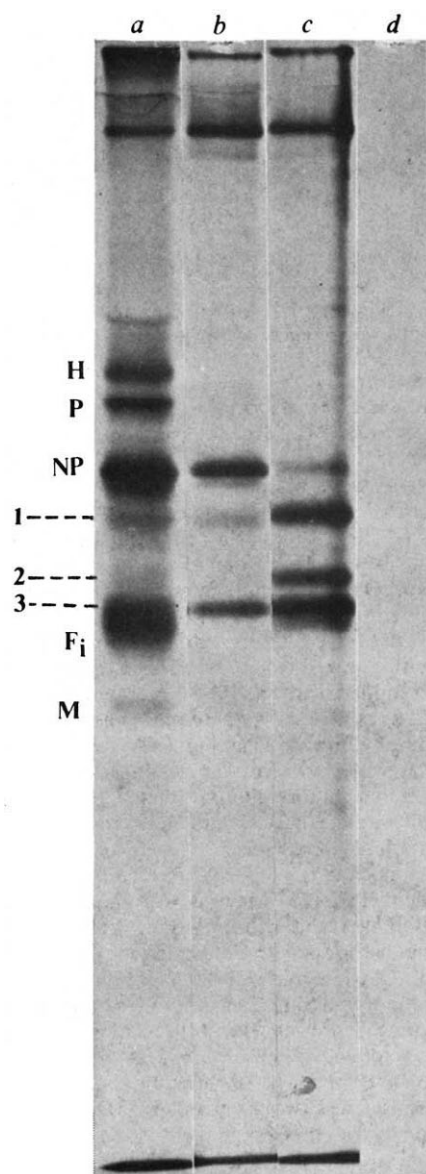


Fig. 2 Polyacrylamide gel electrophoretic analysis of the measles virus polypeptides precipitated by the various monoclonal antibodies. *a*, Virus polypeptides precipitated by convalescent serum of a patient with atypical measles were used as markers. The polypeptides are as follows: H, the virus haemagglutinin; P, a polypeptide associated with the internal nucleocapsid structure; NP, the major structural polypeptide of the nucleocapsid; F, the polypeptide responsible for cell fusion and haemolytic activities; M, the non-glycosylated membrane polypeptide. Bands 1, 2 and 3 are not unique virus polypeptides, but represent proteolytic cleavage fragments of the NP polypeptide⁸. *b*, Virus polypeptides precipitated by antibody D3. *c*, Polypeptides precipitated by antibody C5. *d*, Culture fluid from the human GM 1500 6TG-A1 2 cell line. Culture fluids of *b*, *c* and *d* were concentrated 20-fold by freeze-drying before use. The procedures used in immunoprecipitation, similar to those described elsewhere¹, were as follows: lysates of virus-infected CV1 cells labelled with ³⁵S-methionine were used as antigen. Aliquots (25 µl) were mixed with 100 µl concentrated culture fluid and incubated at 37 °C for 90 min then at 4 °C for 4 h. Rabbit total anti-human antibody (25 µl) was then added and the incubation period repeated. Precipitated polypeptides were collected by centrifugation in an Eppendorf centrifuge for 20 min at 10,000 r.p.m. The visible pellet was resuspended and washed three times. After the final washing, the pellet was suspended in lysis buffer, boiled for 3 min and electrophoresed on a 10% SDS-polyacrylamide gel in conditions described elsewhere¹. After fluorography, dried gels were exposed to Cronex X-ray film.

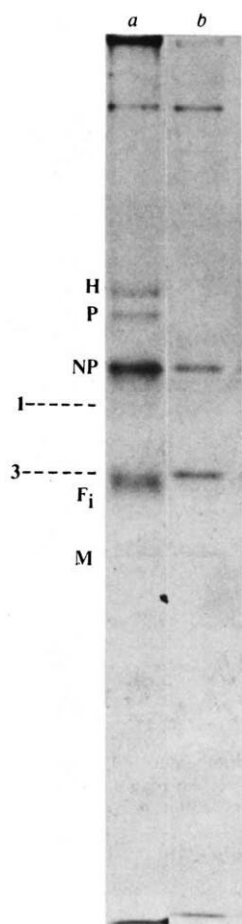


Fig. 3 Lane *a* represents the virus polypeptides precipitated by the same atypical measles serum as in Fig. 2*a*. *b*, Polyacrylamide gel electrophoretic analysis of the measles virus polypeptides precipitated by culture fluid from subclone D3M2. The conditions of the experiments were as described in the legend to Fig. 2, except that culture fluid was unconcentrated.

hypoxanthine-aminopterin-thymidine (HAT)⁶ selective medium (data not shown). The mutant cells were found to secrete human IgG ($\gamma 2$, κ), as did the parental GM 1500 cells (Fig. 1, lane 1)³.

Heparinized blood plasma was obtained from a patient suffering from SSPE. This patient was a 19-yr-old female who had first developed SSPE at age 9—the disease lasted for 2 yr, leading ultimately to recovery with the persistence of only some residual symptoms. At the age of 18 yr, she married and became pregnant, then in the fourth month of pregnancy SSPE recurred with great severity and she is now in a chronic vegetative state. Measles virus was isolated from a brain fragment during the first tissue culture. Serum from the patient, diluted 1:10⁶, bound in radioimmunoassay (RIA) with measles-infected target cells. These data, as well as typical histological lesions of the brain, confirmed the clinical diagnosis of SSPE.

Using established procedures^{3,4}, we fused 10⁷ GM 1500 6TG-A1 2 cells with Ficoll-purified lymphocytes derived from peripheral blood (10 ml) taken from the patient in the presence of polyethylene glycol 1000 (ref. 7). The fused cells were distributed into 24 wells of a Linbro FB-16-24 TC plate in the presence of HAT selective medium. Growth of hybrids was detected in 20 of the 24 wells. These hybrids were propagated in HAT-RPMI 1640 medium and cloned by limiting dilution. Each independent clone (derived from an independent well) was tested for the expression of human immunoglobulin chains and for the ability to immunoprecipitate measles virus proteins⁸.

As shown in Fig. 1, six hybrid clones, including the two that reacted with measles virus (see Fig. 2), expressed the human μ chains, as determined by immunoprecipitation of the immunoglobulins secreted by the hybrids with rabbit anti-human μ antiserum^{3,4}. Some hybridomas also expressed a light chain (L) that migrated differently from the light chain expressed by the GM 1500 line. The clone of the hybridoma illustrated in lane 6 (Fig. 1) lost the ability to produce the light chain expressed by

GM 1500. The hybridoma culture fluid in lane 7 was immunoprecipitated with both anti-human μ and anti-human γ chain antisera—the hybrid expressed two heavy chains, the γ chain of the GM 1500 parent and the μ chain of the human SSPE B-cell parent.

The specificity of the antibodies for measles virus was determined by immunoprecipitation using methods similar to those described elsewhere⁸. Culture fluids from cultures D3 and C5 only precipitated the virus nucleocapsid polypeptide (NP) and varying amounts of its cleavage fragments (Fig. 2). The antibodies in the culture fluids seemed to be specific for this one polypeptide which is the major polypeptide of the virus nucleocapsid⁸. NP is extremely sensitive to proteolytic cleavage⁸, thus the long incubation periods used in the immunoprecipitation procedure could be partly responsible for the high level of cleavage of the polypeptide. The level of cleavage is particularly evident in Fig. 2*c*. The specificity of the antibodies for NP was confirmed by the demonstration that subclones of hybrid clone D3 could also precipitate this polypeptide (Fig. 3*b*).

These results indicate that it is possible to obtain human B-cell hybrids which continuously secrete human antibodies, particularly antibodies specific for a human pathogenic virus. The availability of a human continuous B-cell line with appropriate drug resistance markers represents a breakthrough in work towards the possible application of this technology to human immunotherapy. In addition, lymphocytes from patients with human autoimmune diseases such as myasthenia gravis and Graves' disease could be fused with human B-cell lines to produce hybridomas that secrete the autoantibodies responsible for the disease. Once such monoclonal autoantibodies are available, the production of anti-autoantibodies could provide a cure for patients with autoimmune diseases.

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Surface phenotypes in T-cell leukaemia are determined by oncogenic retroviruses

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Spontaneous thymic leukaemia in experimental mice is the result of a complex series of genetically controlled events¹. An important step in this process involves the production by thymocytes of recombinant polytropic retroviruses (MCF viruses)^{2,3}. These leukaemogenic agents arise by recombination of genes from the *env* regions of endogenous precursor viruses^{4,5}. Sequences in these regions encode the envelope glycoprotein gp70 (ref. 6). Thus far, each cloned isolate of recombinant virus from AKR and HRS/J mice has been found to possess unique oligonucleotide sequences in its *env* region, as well as clone-specific peptides in its gp70 (refs 7, 8). Therefore, the polytropic viruses of these leukaemia-susceptible mice are

extremely diverse. These findings suggest that random recombination of *env* genes gives rise to leukaemogenic polytropic viruses. McGrath and Weissman⁹ have proposed that thymocytes with cell surface receptors for the gp70 of a particular leukaemogenic virus are the target cells for malignant transformation by that specific virus. In view of the diversity of polytropic viral gp70, their hypothesis would predict extensive phenotypic diversity among spontaneous thymic leukaemias. In contrast, leukaemias induced by a particular leukaemogenic recombinant virus would always have the same phenotype. Here we verify these predictions experimentally.

We studied thymomas that arose spontaneously in HRS/J mice, or that were induced in HRS/J or CBA/J mice by neonatal inoculation of cloned polytropic viruses of HRS/J origin⁸. The surface phenotypes of these tumours were characterized by fluorescent staining of the cells with monoclonal antisera and other reagents, followed by analyses on a fluorescence-activated cell sorter (FACS IV, Becton Dickinson). The surface phenotypes of thymocytes obtained from normal HRS/J and CBA/J mice were also studied with these reagents.

The results obtained with normal thymocytes were similar to those already published¹⁰, that is, consistently high Thy-1, low H-2K^k and Ia^k, and consistently high Lyt-1 and Lyt-2 (Table 1). The percentage of cells that stained with fluorescein isothiocyanate (FITC)-conjugated peanut agglutinin (PNA), a marker of immature thymocytes¹¹, was high in all cases. The expression of murine leukaemia virus (MuLV) antigen was low except in old (40-week) HRS/J mice, in which an increase in this antigen is expected³.

In comparison, the surface phenotypes of thymocytes from eight spontaneous HRS/J thymomas had no consistent pattern. The thymic tumours were taken from mice that were chosen at random from our animal colony. The sole criterion for the selection was clinical evidence of a tumour. Figure 1 shows that each tumour had a distinctive phenotype, although several features were shared by all of them. All tumours expressed Thy-1.2, although in two cases (*d* and *h*) the percentage of thymocytes that were positive for this marker was lower than in the other six. Moreover, all the neoplasms contained a high percentage of thymocytes that expressed MuLV antigen. All the

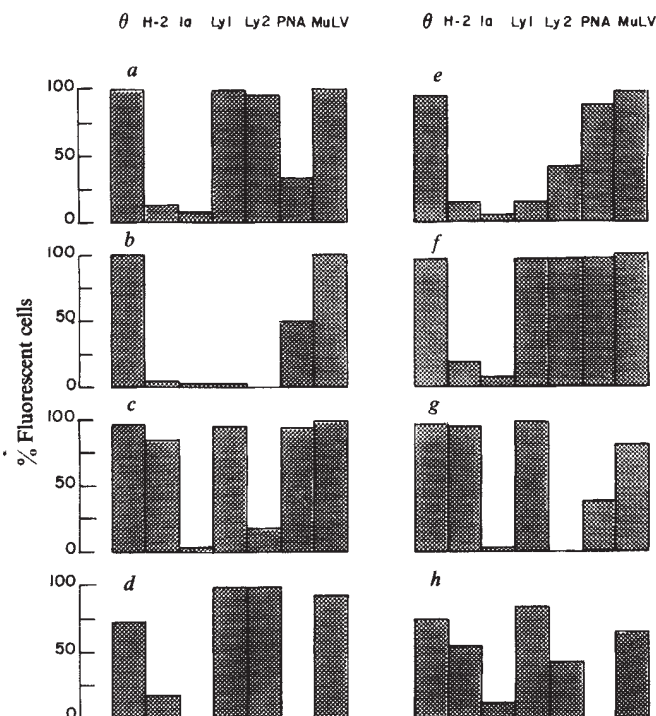


Fig. 1 Thymocyte surface markers of randomly selected spontaneously occurring thymomas in HRS/J mice. All reagents and techniques were the same as for experiments in Table 1. *θ*, Thy-1.2; H-2, H-2K^k; Ia, Ia^k; Lyt-1, Lyt-1; Lyt-2, Lyt-2. Note that each tumour has a distinctive phenotype.

thymomas were low in Ia^k, which may be characteristic of the HRS/J strain because thymomas from AKR mice express increased amounts of this antigen¹². Other surface markers (H-2K^k, Lyt-1, Lyt-2) and the percentage of cells with receptors for PNA varied independently.

The phenotypes of thymomas that were induced by cloned isolates of two HRS/J polytropic viruses (PTV 1 and PTV 2) were examined next. These viruses have been characterized biochemically and each has distinctive oligonucleotides in the *env* region as well as distinctive gp70 peptides⁸. They are highly thymotropic and leukaemogenic in both HRS/J and CBA/J mice⁸. The surface phenotypes of thymomas induced by injection of neonatal mice with PTV 1 or PTV 2 are shown in Table 2. Each cloned virus regularly produced thymomas with phenotypes characteristic of that particular clone. Thus, the neoplasms induced by PTV 1 were low in Thy-1.2, high in H-2K^k, increased in Ia^k, high in Lyt-1 and low in Lyt-2. Expression of MuLV by these cells was always high. The percentage of thymocytes with receptors for PNA varied; four thymomas had many and four had few PNA⁺ cells. In contrast, PTV 2 induced the development of malignant thymocytes with high expression of Thy-1.2, H-2K^k, and Lyt-1, whereas the expression of Lyt-2 and Ia^k was low. MuLV antigen was again expressed by a high percentage of cells. PNA receptor-bearing thymocytes were low in four of five cases. The surface phenotypes of leukaemic cells induced by PTV-1 were similar in CBA, HRS/J and (CBA × HRS/J)_{F1} recipients.

These results demonstrate that the surface phenotypes of spontaneously occurring thymomas are apparently random, whereas the phenotypes of thymomas induced by cloned leukaemogenic viruses are predictable and characteristic of a given clone. The PTV 1- and PTV 2-induced leukaemias produce the specific recombinant virus which induced them⁸, so it is unlikely that a new recombinant virus caused these leukaemias. Pepersack *et al.*¹³ found phenotypic heterogeneity among thymomas induced by Moloney leukaemia virus. Stocks of this agent contain recombinant viruses¹⁴ which might vary

Table 1 Expression of cell surface markers and MuLV antigen by normal CBA/J and HRS/J mice of different ages

Strain	Age (weeks)	Age						MuLV
		Thy-1.2*	H-2K ^k	Ia ^k	Lyt-1	Lyt-2	PNA	
CBA	8	97.7	9.3	1.5	90.0	88.6	82.7	0.8
CBA	20	97.9	8.6	3.2	93.9	89.6	84.9	1.4
CBA	28	97.8	6.5	1.6	86.4	90.0	85.4	0.6
CBA	28	96.7	7.8	1.3	87.0	91.0	85.0	1.0
CBA	40	95.1	8.5	1.9	89.8	90.0	87.6	1.2
CBA	40	85.1	11.0	7.6	92.2	81.2	75.7	4.7
CBA	40	92.4	6.4	2.6	90.3	88.0	87.7	1.7
hr/hr	8	93.5	8.9	3.7	90.5	89.5	—	0.8
hr/+	8	91.8	8.7	5.9	98.8	85.0	—	1.1
hr/+	20	95.7	10.3	4.0	91.2	84.6	—	18.9
hr/hr	24	99.2	14.0	4.1	—	—	—	10.9
hr/hr	28	98.3	16.2	2.9	87.4	81.7	83.8	24.0

Suspensions of thymocytes were prepared in phosphate-buffered saline (PBS) supplemented with 5% heat-inactivated fetal calf serum (iFCS) and 0.1% sodium azide. 1×10^6 thymocytes were incubated with the following monoclonal antisera for 30 min on ice: anti-Thy-1.2 (NEN, dilution 1:1,000), biotinylated anti-H-2K^k (1:50) and anti-Ia^k (1:50), directly fluoresceinated anti-Lyt-1 and -Lyt-2 (Becton-Dickinson; both at a dilution of 3 μ l antiserum per 100 μ l diluent). Also used were FITC-PNA (Pharmindustrial, 1:50), and FITC-goat antiserum prepared against Tween-ether disrupted Moloney MuLV (1:1,000). The cells were washed through FCS and incubated, when necessary, with the appropriate second-step reagent—FITC-rabbit anti-mouse immunoglobulin (1:40) or fluorescein-avidin D (Vector Laboratories, 1:50) for another 30 min on ice. The percentages of fluorescent cells were determined and recorded on a FACS.

* Percentage of fluorescent cells.

from one preparation to another. Studies in AKR/J mice also indicate heterogeneity of spontaneous thymomas¹⁵⁻¹⁷. Thus, these tumours may be considered as an example of genetically determined neoplasms with a randomly specified phenotype. The diverse phenotypic patterns of these spontaneous leukaemias are consistent with the concept that the causative leukaemogenic viruses arise by random genetic recombination¹⁸.

The results with cloned viruses demonstrate the induction of neoplasms with virus-specific phenotypes. The mechanism whereby cloned viruses induce leukaemias with a specific phenotype is unknown, but a reasonable conjecture is that this process is related to gp70 because the only major difference between PTV 1 and PTV 2 is in the structure of this glycoprotein⁸. Elder *et al.*¹⁸ have proposed that gp70 may be a transforming protein. We think it unlikely that gp70 contains the information that would be required to specify the distinctive and characteristic leukaemia cell phenotypes we observed. Alternatively, the type of cell that is programmed to undergo transformation by a given retrovirus might be selected by the fortuitous affinity of its receptor for the gp70 of that virus. This hypothesis predicts that certain recombinant retroviruses will not be leukaemogenic because their gp70 cannot bind with sufficient affinity to the receptor of any thymocyte. Moreover, genetic variants of these receptors would explain why a particular recombinant virus is leukaemogenic in some inbred strains of mice, but not in others^{7,8}. McGrath and Weissman⁹ have argued that recombinant viral gp70 acts via such receptors to stimulate thymocytes immunologically. This persistent immunological stimulation, they propose, leads to malignant transformation of the cell. Their concept implies that the receptor for gp70 is antigen-specific and encoded by V_H genes. Whereas experimental evidence supports the idea that persistent immunological stimulation can lead to lymphoid malignancy¹⁹, the receptors being discussed need not have an immunological function. These hypothetical receptors may act in leukaemogenesis simply as points of attachment and penetration by the leukaemogenic agent. If the thymomas we studied represent neoplastic counterparts of various stages of thymocyte differentiation, then the receptors may be differentiation structures on the cell surface rather than antigen-specific receptors.

Table 2 Surface phenotypes of thymic tumours that developed after intraperitoneal injection of newborn mice with recombinant viruses PTV 1 and PTV 2

Strain	Thy-1.2*	H-2K ^k	Ia ^k	Lyt-1	Lyt-2	PNA	MuLV
a PTV 1							
CBA	75.6	80.4	17.7	88.6	22.3	82.0	82.5
CBA	80.1	86.6	16.0	84.3	7.3	64.6	90.1
HRS	77.7	98.8	8.1	97.0	3.5	37.0	88.4
HRS	—	98.7	11.3	99.1	4.4	98.7	98.6
HRS	—	85.3	9.3	96.6	7.5	44.0	99.1
(CBA × HRS)F ₁	86.1	98.0	12.0	97.1	39.6	94.1	51.1
(CBA × HRS)F ₁	52.2	96.8	32.8	97.1	4.5	98.2	99.2
(CBA × HRS)F ₁	75.0	96.0	9.0	94.6	19.6	19.6	70.0
b PTV 2							
CBA	98.5	76.2	27.1	89.0	25.9	9.1	96.7
(CBA × HRS)	98.4	78.4	3.0	88.9	4.2	26.9	83.6
(CBA × HRS)F ₁	98.6	74.9	2.0	84.0	1.9	19.0	60.3
(CBA × HRS)F ₁	95.0	87.0	6.6	90.0	9.0	84.1	93.1
(CBA × HRS)F ₁	99.1	65.3	1.5	95.5	3.5	7.4	71.5

In the group injected with PTV 1 were two CBA, three HRS/J and three (CBA × HRS)F₁ mice (the latter were bred in our animal facility), and in the group injected with PTV 2 were one CBA and four (CBA × HRS)F₁ mice. The viruses were isolated, cloned and characterized according to previously described methods⁸. The latent period between virus injection and the occurrence of thymoma, and the incidence of thymomas did not differ from previous observations⁸. The dose of virus administered to the mice was about 1×10^5 focus-forming units. All reagents and techniques were the same as for experiments in Table 1.

* Percentage of fluorescent cells.

We cannot exclude the possibility that the clones of viruses we studied possess a relevant transforming function that has not been revealed by the previous biochemical analyses of their genomes and major structural proteins. With certain oncogenic avian retroviruses, for example, transforming genes that are specific for certain types of haematopoietic cells have been demonstrated²⁰. Clarification of the mechanisms whereby cloned recombinant retroviruses induce characteristic clones of leukaemic cells is therefore in progress. Finally, note that the diversity of cell-surface phenotypes in mouse T-cell leukaemias of viral aetiology, as shown here, resembles a similar heterogeneity in human T-cell leukaemia²¹.

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Is myosin in the cochlea a basis for active motility?

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Recent evidence suggests that the stereocilia of cochlear hair cells contain actin filaments¹⁻³. The experiments reported here demonstrate the presence of myosin, suggesting that the mechanical properties of the hair cells may be actively modifiable by actin-myosin interactions as in muscle cells.

Myosin was detected in the hair cells of the guinea pig cochlea by indirect immunofluorescence using an antiserum raised against human smooth muscle myosin⁴. Immunofluorescence was visible in very specific areas of the organ of Corti. The stereocilia fluoresced strongly along their length (Fig. 1a). It is possible that the bright spots of fluorescence seen in one row of stereocilia in Fig. 1a indicate a concentration of myosin near the roots of the stereocilia, perhaps due to the many rows of short

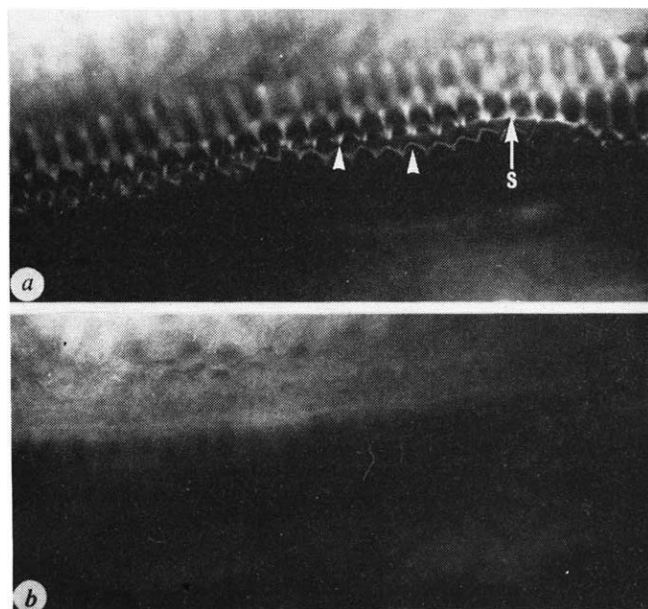


Fig. 1 Anti-myosin immunofluorescence (a), and control section using myosin-absorbed antiserum (b) in the outer hair cells of the guinea pig cochlea, seen from above ($\times 512$). The stereocilia of two rows of outer hair cells are in focus at different depths (white arrows, a). Fluorescence is also seen in the apical ends of the supporting cells, in between the hair cells (S). Guinea pig cochleas were fixed briefly in buffered formalin glutaraldehyde⁹ and the organ of Corti rapidly dissected out and washed in tris saline buffer (pH 7.6). The intact organs of Corti were fixed in cold acetone (-10°C) for 10 min followed by brief air drying and further washing in buffer. Following removal of the buffer the organs of Corti were incubated in a rabbit antiserum raised against smooth muscle myosin⁴ diluted 1:20 with tris saline buffer for 60 min, followed by washing in three changes of buffer. The second stage antiserum was a fluorescein-labelled swine anti-rabbit immunoglobulin (Dako-Immunoglobulins 52190) diluted 1:20 in buffer. After washing the specimens were orientated and mounted in glycerol-phosphate buffered saline. They were viewed using a Leitz SM-Lux microscope fitted with a fluorescence vertical illuminator for incident light excitation. Controls included the use of preimmune rabbit serum and myosin-absorbed antiserum in place of the first stage anti-myosin serum.

stereocilia. A similar pattern of fluorescence was seen in inner and outer hair cells. In transverse sections faint immunofluorescence was also seen in the cuticular plate at the apex of the hair cells. Therefore in contrast to the microvilli of the intestinal brush border, most of the immunofluorescence was associated with the cilia rather than any terminal web. It is possible that actin-myosin interactions in such areas could lead to a shortening or a change in stiffness of the stereocilia. Although there is little information on the amount of movement possible, the results of Tilney *et al.*² strongly suggest that it may be limited, perhaps due to the large numbers of cross-bridges between the actin filaments³. In both transverse and surface preparations immunofluorescence was also visible in the supporting cells between the apical ends of the hair cells (Fig. 1a). It is very unlikely that such structures deform, even passively, thus although these experiments indicate that the molecular basis of actin-myosin interactions exists in the cochlea, they do not indicate that motility must necessarily occur.

These results have the following implications: (1) it has been suggested that the cochlear transducer can produce sound⁵, which may be the result of actin-myosin motility; (2) the changes in the mechanical properties of the cochlea seen after death⁶ or the loss of rigidity of the stereocilia, which is an early sign of sound-induced damage⁷, may result from changes in the actin-

myosin linkage; (3) similarly, in physiological conditions the stereocilia may not be as stiff as demonstrated by Flock¹ in the excised organ; (4) Ca^{2+} , which is essential for the transduction process⁸, may enter the stereocilia as a result of sound stimulation and so affect the mechanical properties of the hair cells; (5) the nonlinear growth of basilar membrane movement with stimulus intensity⁶ may be due to the progressive activation of the actin-myosin linkage as a result of sound stimulation; and (6) the fibres of the olivocochlear bundle may affect the mechanical properties of the hair cells by altering the actin-myosin linkage, perhaps as a result of changed Ca^{2+} influx.

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Ca^{2+} -activated ATPase and ATP-dependent calmodulin-stimulated Ca^{2+} transport in islet cell plasma membrane

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Calcium is known to play an essential part in the regulation of insulin secretion in the pancreatic β cell^{1,2}. Calcium influx/efflux studies indicate that glucose promotes an accumulation of calcium by the β cell². However, interpretation of such data is particularly difficult due to the complex compartmentalization of calcium within the cell. Although indirect evidence using chlorotetracycline suggests that control of calcium homeostasis at the plasma membrane may be central to insulin secretion³, the mechanism by which secretagogues influence the handling of calcium remains unknown. Despite its continuous diffusive entry, intracellular calcium is maintained in the submicromolar range^{4,5} by energy-dependent mechanisms⁶⁻⁹. One such process which has been well characterized in erythrocytes is a plasma membrane calcium extrusion pump whose enzymatic basis is a high affinity ($\text{Ca}^{2+} + \text{Mg}^{2+}$)ATPase⁹. A similar mechanism^{10,11} regulated by insulin^{12,13} has recently been identified in adipocyte plasma membranes. We report here the presence of a high affinity ($\text{Ca}^{2+} + \text{Mg}^{2+}$)ATPase and ATP-dependent calmodulin-stimulated calcium transport system in rat pancreatic islet cell plasma membranes¹⁴.

Calcium-stimulated ATPase activity was demonstrated in the plasma-membrane enriched fraction from pancreatic islets. The dependence of ATPase activity on calcium concentration revealed the presence of two saturable components similar to those observed in erythrocyte¹⁵ and adipocyte¹⁰ plasma membranes. One had a high affinity for calcium, designated ($\text{Ca}^{2+} + \text{Mg}^{2+}$)ATPase, and achieved saturation at submicro-

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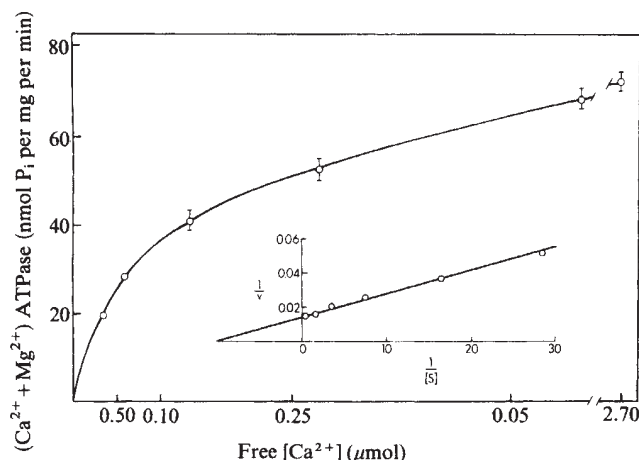


Fig. 1 Calcium dependence of the high affinity ($\text{Ca}^{2+} + \text{Mg}^{2+}$)ATPase in pancreatic islet cell plasma membranes. Islets were isolated from male Sprague-Dawley rats by the method of Lacy and Kostianovsky³⁷ and the plasma membrane-enriched fraction (band I) was obtained by the method of Naber *et al.*^{20,43} with the modification that EDTA was omitted from the homogenization and fractionation buffers. Omission of EDTA does not modify the distribution of organelles among the subcellular fractions^{20,43}. The plasma membranes were frozen immediately and were used within 2 days of storage at -70°C . ($\text{Ca}^{2+} + \text{Mg}^{2+}$)ATPase activity was quantitated by monitoring the release of $^{32}\text{P}_i$ from $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and is expressed as nmol P_i per mg protein per min (ref. 38). Protein was determined as described elsewhere³⁹. The assay medium contained 1–2 μg protein per ml with 2 mM EGTA, 1.4–2 mM CaCl_2 (equivalent to 0.035–2.7 μM free Ca^{2+}), 1–2 μCi $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, 0.25 mM ATP (Tris salt), 20 mM NaN_3 and 10 mM Tris-PIPES, pH 7.5 at 37°C . The incubation volume was 1.0 ml. Reaction times were 30 or 60 min. The ATPase reaction rates were linear over the assay period and directly proportional to the amount of tissue added. The amount of ATP hydrolysed was always less than 5% of the total ATP present over the incubation period. Calcium-stimulated activity was determined by subtracting values obtained with chelator alone (background activity) from those obtained with calcium plus chelator. The background ATPase was $18 \pm 4\%$ ($n=6$) of the maximum velocity of the high affinity ($\text{Ca}^{2+} + \text{Mg}^{2+}$)ATPase. Total endogenous calcium and magnesium in the assay medium were 2.0–2.5 and 8.5–9.0 μM , respectively, as measured by atomic absorption spectroscopy. Each point represents the mean $\pm$ s.e.m. of triplicate determinations. The inset represents a double-reciprocal plot of the data. Free calcium was calculated using an apparent Ca-EGTA association constant of $10^{7.832} \text{ M}^{-1}$ which was determined for pH 7.5 as described by Portzehl *et al.*⁴. The high affinity ($\text{Ca}^{2+} + \text{Mg}^{2+}$)ATPase was studied at free calcium concentrations $< 2.7 \mu\text{M}$ —in these conditions ATP has a negligible effect on free calcium in a Ca-EGTA buffer system because the apparent Ca-ATP association constant ($10^{3.390} \text{ M}^{-1}$ at pH 7.5) is four orders of magnitude less than that of Ca-EGTA. All cation-ligand association constants were obtained from Sillén and Martell²⁴.

molar concentrations of free calcium (Fig. 1). Data from five separate preparations yielded a K_m of $0.093 \pm 0.010 \mu\text{M}$ free calcium and a Hill coefficient of 1.5 ± 0.2 , both similar to values obtained for erythrocyte¹⁶ and adipocyte¹⁰ plasma membranes. The value of $V_{\max}$ was 54.8 ± 10.1 nmol of P_i released per mg protein per min. The second saturable component which was observed above 10 μM had a comparatively low affinity for free calcium (data not shown).

It is important to demonstrate that the ($\text{Ca}^{2+} + \text{Mg}^{2+}$)ATPase reported here originates from the plasma membrane, because the endoplasmic reticulum of other tissues (and therefore probably the β cell) has a high affinity ($\text{Ca}^{2+} + \text{Mg}^{2+}$)ATPase^{17,18} which also drives a calcium pump^{18,19}. Three lines of evidence support a plasma membrane location: first, the distribution of the high affinity ($\text{Ca}^{2+} + \text{Mg}^{2+}$)ATPase amongst the four islet-

cell subcellular fractions was shown to parallel directly the distribution of the plasma membrane-specific marker enzyme 5'-nucleotidase with a highly significant degree of correlation (Fig. 2). Second, the ($\text{Ca}^{2+} + \text{Mg}^{2+}$)ATPase activity was inversely related to the distribution of NADH cytochrome *c* reductase (an endoplasmic reticulum-specific marker enzyme) amongst the four subcellular fractions^{20,43} and third, both endoplasmic¹⁷ and sarcoplasmic²¹ reticulum ($\text{Ca}^{2+} + \text{Mg}^{2+}$)ATPases from other cells are strongly stimulated by potassium ($K_m = 10 \text{ mM}$), whereas the ($\text{Ca}^{2+} + \text{Mg}^{2+}$)ATPase reported here was slightly inhibited ($17 \pm 5\%$, $n=3$) by 20 mM potassium. Also, as in the erythrocyte⁹ and adipocyte¹⁰, 0.8 mM ouabain had no effect on the ($\text{Ca}^{2+} + \text{Mg}^{2+}$)ATPase.

A calcium-activated ATPase has been identified in subcellular fractions from mouse pancreatic islets by Formby *et al.*²². They reported a high affinity activity ($K_m = 0.18 \mu\text{M}$) which seems similar to the high affinity enzyme reported here. However, there is no reason to believe that their high affinity activity represents a plasma membrane enzyme because it is detectable in all subcellular fractions. Indeed, the authors suggested that mitochondria and secretory granules were mostly responsible for their high affinity Ca^{2+} -ATPase²², but it is possible that it originated from a plasma membrane contamination of the subcellular fractions. We also observed a small inhibitory effect ($17 \pm 6\%$, $n=3$) of sodium (20 mM NaCl) similar to that observed by Formby *et al.*²² at high ATP concentration. In these experiments 1 mM dicyclohexycarbodiimide instead of 20 mM sodium azide was used to block the mitochondrial ATPase²³.

Although magnesium *per se* was not added to the assay medium, it seemed to be necessary for activity, a requirement apparently common to all ion-transporting enzymes. This was shown using the chelator, cyclohexane-1,2-diamine-*N,N,N',N'*-tetraacetic acid (CDTA, Sigma) which has high affinities for calcium and magnesium whereas EGTA has a high affinity for calcium alone²⁴. In the Ca^{2+} -CDTA buffer system, $>99\%$ of the magnesium present in the assay medium (8.5–9.0 μM as measured by atomic absorption spectroscopy) was complexed. When free calcium was maintained at 0.283 μM

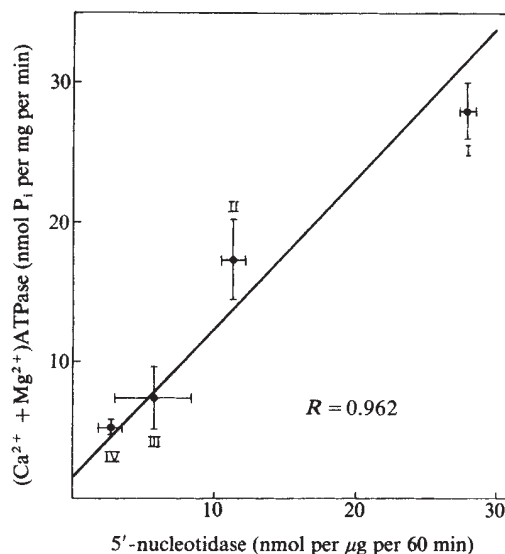


Fig. 2 Distribution of the high affinity ($\text{Ca}^{2+} + \text{Mg}^{2+}$)ATPase relative to 5'-nucleotidase amongst pancreatic islet subcellular fractions. The plasma membrane-specific marker enzyme, 5'-nucleotidase⁴⁰, was assayed by the method of Avruch and Wallach⁴¹. Bands I, II, III and IV as designated by Naber *et al.*^{20,43} are shown. Free $[\text{Ca}^{2+}] = 0.283 \mu\text{M}$. Other assay conditions were as described in Fig. 1 legend. Each point represents the mean $\pm$ s.e.m. of triplicate determinations for both enzyme activities. Band I, which had the highest activities of both enzymes, was the plasma membrane-enriched fraction used throughout these studies.

using this system, 98% of the $(\text{Ca}^{2+} + \text{Mg}^{2+})\text{ATPase}$ activity was lost as compared with the activity obtained in a Ca^{2+} -EGTA buffer at the same free calcium concentration. Therefore, like the adipocyte enzyme¹⁰, both calcium and magnesium seem to be required simultaneously for high affinity $(\text{Ca}^{2+} + \text{Mg}^{2+})\text{ATPase}$ activity. Calcium or magnesium alone cannot produce significant ATPase activity below 10 μM .

$(\text{Ca}^{2+} + \text{Mg}^{2+})\text{ATPase}$ activity gave a complex relationship with varying ATP concentration. Two apparent saturable components with low ($K_{0.5} = 2.1 \mu\text{M}$) and high ($K_{0.5} = 70 \mu\text{M}$) affinities were observed, and were obtained by fitting the Hill equation to the data using the computer program developed by Atkins²⁵. (The estimated V_{max} for the high affinity component was subtracted from the total activity to obtain data for the low affinity component.) Similar observations have been reported for the high affinity $(\text{Ca}^{2+} + \text{Mg}^{2+})\text{ATPases}$ of erythrocyte^{26,27} and adipocyte¹⁰ plasma membranes.

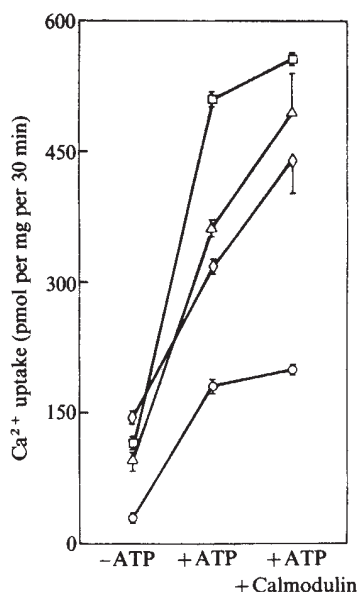


Fig. 3 Calmodulin stimulation of ATP-dependent calcium uptake in pancreatic islet cell plasma membranes. Transport was assayed as uptake of $^{45}\text{Ca}^{2+}$ from a medium containing: 200 mM sucrose, 20 mM NaNO_3 , 2.5 mM MgCl_2 , 1.5 mM Na_2ATP , 10 mM Tris-oxalate, 2 mM Tris-EGTA, 1.8 mM CaCl_2 (0.134 μM free $[\text{Ca}^{2+}]$), 4–8 μCi $^{45}\text{CaCl}_2$ and 10 mM Tris-PIPES, pH 7.5 at 37 °C. Magnesium (2 mM) was added to the transport assay medium because it seems to be necessary for ATP-coupled calcium transport²⁷. The reaction was initiated by addition of 10–15 μg tissue and incubated for 30 min at 37 °C. The incubation volume was 0.5 ml and the reaction was terminated by rapid filtration of a 0.4-ml aliquot on 0.45- μm Millipore HAWP filters and washed with 30 volumes of isotonic sucrose. Calcium uptake is shown in the absence (far left) and presence of ATP (middle) and with ATP plus calmodulin (far right). Uptake was not linear with time but was directly proportional to the amount of tissue added and is reported as the level attained after 30 min. The presence of ATP and/or calmodulin did not affect the amount of radioactivity bound to the filters. Furthermore, calmodulin did not affect the amount of $^{45}\text{Ca}^{2+}$ associated with the membranes in the absence of ATP. The data represent results from four separate experiments and are presented as the mean $\pm$ s.e.m. Each symbol type represents a different preparation. Purified calmodulin was prepared from Sprague–Dawley rat brains by a previously described method⁴² and was added to a final concentration of 43 nmol (0.72 $\mu\text{g ml}^{-1}$) assuming a molecular weight of 16,723 (ref. 36). Purity of the calmodulin preparation was established by resolution into a single homogeneous band by SDS-polyacrylamide gel electrophoresis. The ability to stimulate calmodulin-dependent cyclic nucleotide phosphodiesterase and erythrocyte plasma membrane $(\text{Ca}^{2+} + \text{Mg}^{2+})\text{ATPase}$ were the criteria used to establish the protein's identity³⁶. The preparations used in transport experiments contained EDTA in the homogenization buffers. The chelator was required during fractionation to dissociate endogenous calmodulin from the membranes³⁵.

To ascertain whether or not the high-affinity plasma membrane $(\text{Ca}^{2+} + \text{Mg}^{2+})\text{ATPase}$ served as the enzymatic basis of an active transport system, we assayed the preparation for $^{45}\text{Ca}^{2+}$ transport. ATP-dependent uptake was observed in the islet cell plasma membranes (Fig. 3) and seems to represent accumulation of calcium in vesicles spontaneously formed during fractionation. The ATP-dependent component does not seem to represent binding of calcium to protein or lipids²⁸ as it was abolished when the vesicles were made freely permeable to calcium with ionophore A23187 (2 μM)²⁹. In this system, the presence of azide excluded any possible participation of energy-dependent sequestration of calcium by contaminating mitochondria^{30,31} and the persistence of calcium uptake activity in the absence of potassium suggests that uptake is not derived from the endoplasmic reticulum, which has been shown in adipocytes to be potassium dependent¹⁹.

The calcium-dependent regulatory protein, calmodulin, which stimulates ATP-dependent calcium uptake across red cell³² and adipocyte¹¹ plasma membranes, had a similar effect in pancreatic islet cell plasma membrane vesicles (Fig. 3). In each of four experiments, calmodulin produced a significant ($P < 0.05$ in a paired t -test) stimulation of ATP-dependent uptake (Fig. 3), ranging from 10 to 74% with a mean of $36 \pm 15\%$. Evaluation of a direct effect of calmodulin on the $(\text{Ca}^{2+} + \text{Mg}^{2+})\text{ATPase}$ may require the presence of millimolar concentrations of magnesium (H.A.P. and J.M.McD., unpublished observations). Interpretation of the data is therefore complicated by the low affinity magnesium-stimulated ATPase and we are now investigating procedures to resolve this problem.

The demonstration of this high affinity $(\text{Ca}^{2+} + \text{Mg}^{2+})\text{ATPase}$ associated with a calmodulin-stimulated active calcium transport system in pancreatic islet cell plasma membranes implies the presence of a mechanism capable of fine control of cytoplasmic free calcium concentration at the level of the plasma membrane. Calmodulin, which is present in pancreatic islets³³, has been shown to activate adenylate cyclase^{34,35} in this tissue, a mechanism which may serve to amplify the secretory response³⁴. Activation of a calcium extrusion pump by calmodulin would provide a powerful means of negative feedback control of calcium and calcium plus calmodulin mediated effects on intermediary metabolism. Studies with intact islets using the calmodulin inhibitor, trifluoperazine, have suggested that calmodulin may play an important part in insulin secretion³³. However, calmodulin regulates many diverse cellular metabolic events³⁶, and thus its exact role in insulin secretion remains to be determined.

Whether or not the $(\text{Ca}^{2+} + \text{Mg}^{2+})\text{ATPase}/\text{Ca}^{2+}$ pump complex is responsible for increased levels of cellular calcium in response to glucose stimulation is unknown, but this possibility can be easily tested using the model system described here. This cell-free system may provide a means of investigating the effects of other secretagogues and agents on calcium homeostasis at the level of the plasma membrane.

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Decamethonium and hexamethonium block K^+ channels of sarcoplasmic reticulum

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The sarcoplasmic reticulum membrane (SR) of skeletal muscle contains cation-selective channels which have been detected by isotope fluxes in fragmented SR vesicles¹, fluorimetric dyes² and direct incorporation of SR vesicles to planar phospholipid bilayers^{3–5}. SR channels incorporated in bilayers have a single open-state conductance of 140 pS in 0.1 M K^+ (refs 4, 5). We have previously reported blockade of the SR channel by Cs^+ , a low-affinity blocker with a zero-voltage dissociation constant of 40 mM (ref. 6). We showed that increasing Cs^+ concentrations reduced the open-channel conductance, increased the mean open time and conferred voltage dependence on the open-state conductance⁶. Here we report on the blockade induced by the cholinergic drugs decamethonium and hexamethonium⁷ on the SR channel. Although blockade by hexamethonium is similar to that of Cs^+ , decamethonium blocks with a much higher affinity and induces flickering events which are probably due to the interaction of single drug molecules with the open state.

Figure 1 shows oscilloscope traces of single channel openings at -40 mV for different concentrations of blocker. Addition of 10 μ M decamethonium induces flickering noise that is observed only when the channels are open. Higher concentrations increase the flickering rate, until at very high concentrations the flickering becomes faster than the amplifier time response; this results in an apparent reduction in the open-state conductance. At 300 μ M decamethonium this time-averaged conductance is about 10% of the control value. Figure 1 also shows that channels blocked with hexamethonium do not flicker, but rather undergo a smooth reduction in open-state conductance as drug concentration is increased. The amount of blocker necessary to reduce the open-state conductance to 50% of its original value is

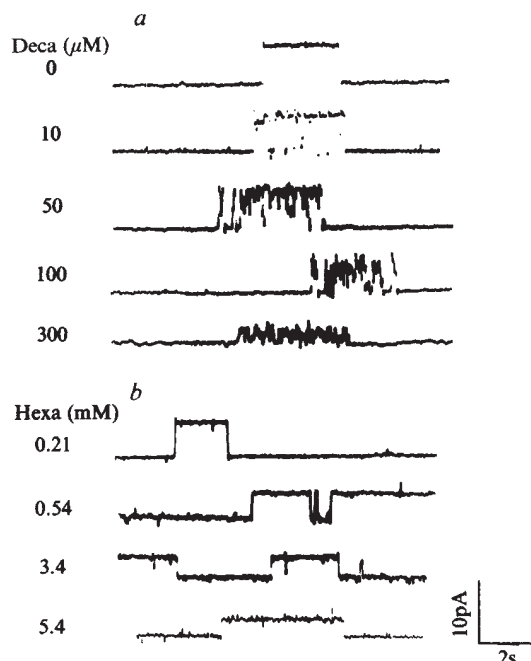


Fig. 1 Lipid bilayers were formed on polystyrene cups with 0.5-mm diameter holes using a 30 mM solution of Soy PE in decane. The aqueous phase was composed of symmetrical solutions of 50 mM K_2SO_4 buffered with 1 mM HEPES-Tris/10 μ M EDTA, pH 7.2. Electrical measurements were made in voltage-clamp conditions using a Burr-Brown 3528CM amplifier. Output current was filtered using a low-pass active filter (Khron-Hite 3202) set at 20 Hz in all experiments except in the fast traces of Fig. 2c, where the cutoff was at 200 Hz. Current traces were taken from a storage oscilloscope and simultaneously recorded on chart paper. The *trans* chamber (side opposite to where SR vesicles are added) is defined as zero voltage. Incorporation of single SR vesicles was accomplished as described elsewhere^{4,5}. Hexamethonium, $(CH_3)_3N^+-(CH_2)_6-N^+(CH_3)_3$ and decamethonium, $(CH_3)_3N^+-(CH_2)_{10}-N^+(CH_3)_3$ (bromide salts, Sigma) were added as glucuronate salts to the *trans* chamber in 50 mM K_2SO_4 buffer. *a*, Traces of individual opening events at -40 mV from the same membrane and at the concentrations of *trans* decamethonium (Deca) indicated. *b*, Channel openings from the same membrane at -40 mV and concentrations of hexamethonium (Hexa) indicated. Experiments were done at room temperature, 21–22 °C.

120 μ M for decamethonium and 3.5 mM for hexamethonium at -40 mV. Three important features of the block induced by decamethonium are shown in Fig. 2. Figure 2a shows that the flickering is voltage dependent. Flickering is observed only at voltages that drive the drug inside the channel (negative voltages). Figure 2b shows that flickering does not interfere with the gating process (that is, the voltage dependence of the open state^{4,5}). A positive voltage was applied to open the channels and then returned to -50 mV to observe the closing events. Repetitive flickering is observed but channels are still able to close at voltages that make the open state less probable^{4,5}. The higher rate of flickering observed in Fig. 2 when five channels are open and its decrease as channels close strongly suggest that the drug blocks each channel independently. In Fig. 2c individual flickering events are displayed on a faster time scale. Traces were taken while a single channel was open. In the conditions specified the mean open time for this experiment was 3.5 s and the mean closed time 18–20 s. Addition of 20 μ M decamethonium segments the open state into much shorter dwell times (~ 200 ms). The individual flickering events, when they could be resolved, were square pulses that dropped the conductance from its open state value to zero and lasted less than 200 ms (see Fig. 2 legend).

We have recently concluded that conduction of cations through the SR channel occurs by movement of the ions across

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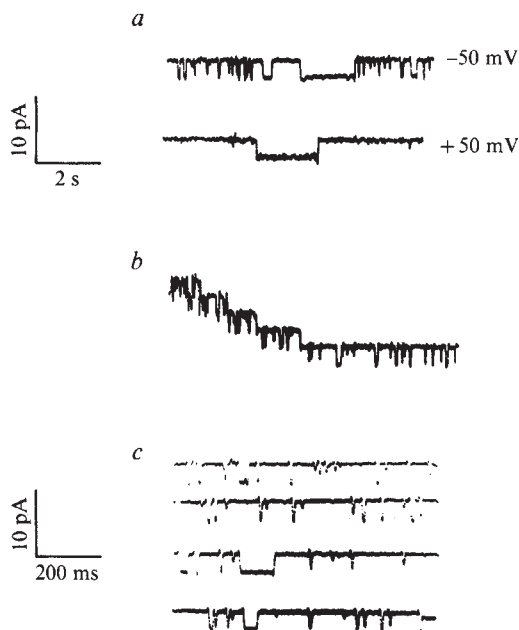


Fig. 2 Channels at low temperature in the presence of 20 μ M decamethonium added *trans*. Temperature was maintained at 7 °C using an ice-water bath. *a*, Recordings from the same membrane at -50 and +50 mV. *b*, Closing events at -50 mV after channels were open by holding the voltage at +50 mV (refs 4, 5). *c*, Recordings while a single channel opens at -50 mV and undergoes repetitive flickering. The mean open time of the open state in this experiment was 3.5 s and the mean closed time was 19.3 s (measurements done before addition of drug). The conductance drop during individual blocking events, measured in the cases where they could be resolved, was 72 ± 2 pS ($n = 10$) whereas the open-state conductance, measured in between flickering, was 71 ± 0.5 pS ($n = 20$). Blocking events were defined as apparent 'closed' states lasting less than 100 ms. These short-lived "closed" dwell times were observed only when the drug was present.

three kinetic barriers and that the channel can accommodate at most one ion at a time (two sites, three barriers, one-ion Eyring model)¹². The one-ion condition implies that the channel can accommodate a blocker ion or a conducting ion but not both simultaneously. Thus, we propose that the short-lived non-conducting dwell times observed in the presence of decamethonium correspond to blocking events generated by single drug molecules as they bind to the conducting channel. Flickering would then be the consequence of the reversible equilibrium of the blocking reaction. A one-site one-blocker model predicts that the time-averaged open-state conductance should decrease in the presence of the drug and that this average should be voltage dependent^{6,9-11}.

An expression that combines the single-site binding with the voltage dependence of the dissociation constant is given by equation (1) shown in Fig. 3 legend. In Fig. 3 we measured the time-averaged conductance $\langle \gamma \rangle$ as a function of voltage at constant and symmetrical concentration of blocker. Starting from the unblocked open-state conductance of 140 pS at positive voltages, the conductance falls steeply at negative voltages for both hexamethonium and decamethonium. Thus, these drugs block only from the *trans* side of the membrane. The solid lines correspond to equation (1) with $K_0 = 11$ mM, $z\delta = -0.66$ for hexamethonium and $K_0 = 1.2$ mM, $z\delta = -1.22$ for decamethonium (see Fig. 3 legend for explanation of symbols). The fit is consistent with the one-site blocking model within the voltage range measured.

The qualitative difference between the two blockers (flickering compared with 'quiet' channels) would be attributable to differences in the lifetime of the blocked state. If a blocker molecule forms blocked states that are short lived with respect to

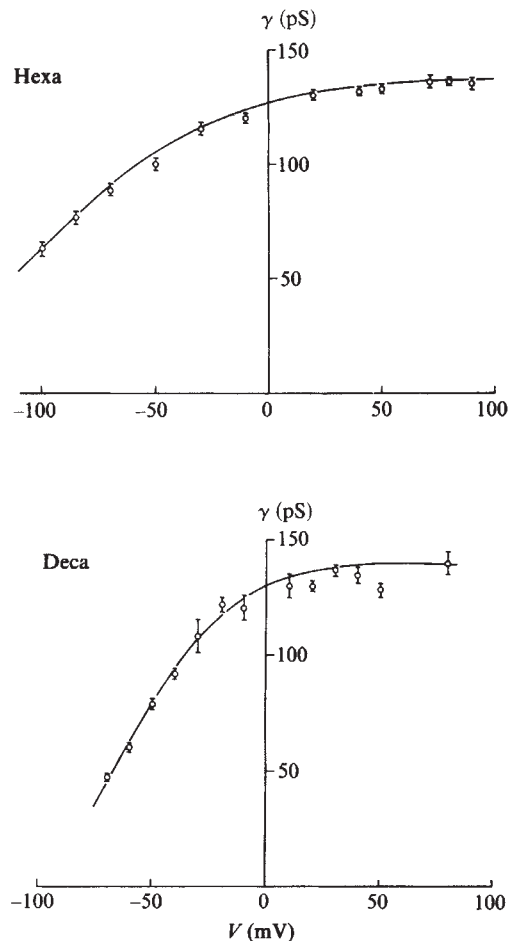


Fig. 3 Voltage-dependent single-channel conductance in symmetrical solutions of 50 mM K_2SO_4 buffer plus 100 μ M decamethonium or 1 mM hexamethonium. To obtain reliable measurements of the time-average conductance of channels with decamethonium, flickering was filtered using the amplifier with a time resolution of 0.5 s. This allowed measurement of a smooth d.c. value for the average open-state conductance. A total of 169 determinations from 4 membranes for decamethonium and 135 determinations from 7 membranes for hexamethonium were used to fit the curves. Bars indicate s.e.m.; the solid lines correspond to the single-site blocking equilibria^{6,9} described by equation (1) below and the parameters given in the text

$$\langle \gamma \rangle / \gamma_0 = [1 + (B/K_0) \exp(z\delta FV/RT)]^{-1} \quad (1)$$

Where $\langle \gamma \rangle$ is the time-averaged conductance in the presence of constant blocker at concentration B ; $\gamma_0 = 140$ pS is the unblocked open-state conductance; K_0 is the dissociation constant of the blocking reaction at zero volts; z is the valence of the blocking ion; δ is the fraction of the total electric potential drop across the membrane found at the blocking site; V is the applied voltage; R , T , F have their usual meanings. Parameters for the fitted curves were obtained from a linearized form of equation (1) as described previously⁶. These parameters are $K_0 = 11 \pm 0.5$ mM, $z\delta = -0.66 \pm 0.02$ for hexamethonium and $K_0 = 1.2 \pm 0.2$ mM, $z\delta = -1.22 \pm 0.08$ for decamethonium.

the time response of the amplifier (5 ms), we would observe only an average apparent open-state conductance equal to the time-average of true open and true blocked conductances¹¹. This seems to be the case for hexamethonium and caesium⁶ in this channel. Molecules forming long-lived blocked states would generate flickering of the open-state conductance. This seems to be the case for lidocaine derivatives in the acetylcholine-receptor channel¹⁰, for tetraalkylammonium ions in the amphotericin channel⁸, and for decamethonium, as described here.

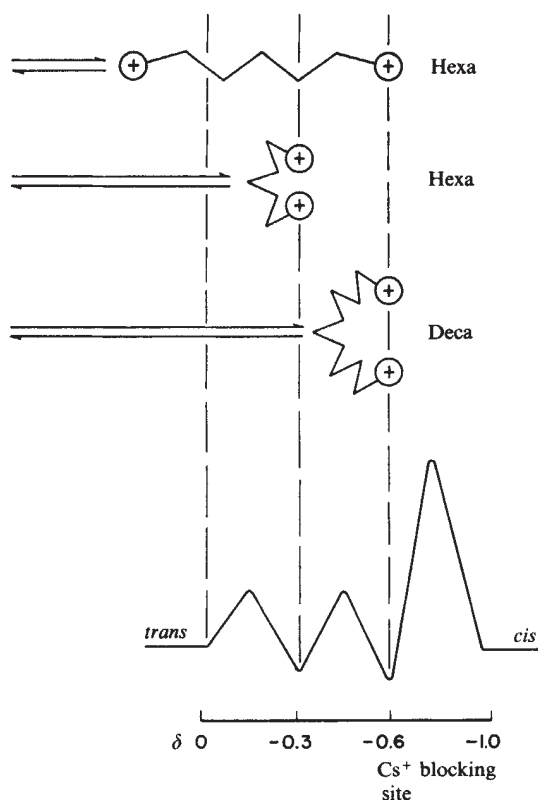


Fig. 4 One or two blocking site models for methonium derivatives. In the same spirit of the two-site conduction model proposed for the SR channel recently¹², we propose that both molecules can block at the Cs^+ site but in different conformations to account for $z\delta$ values measured (upper part). The alternative, of block at different sites, implies that hexamethonium would also act in a bent conformation. We envision that for methonium molecules at least one of the energy barriers (the *cis*-facing barrier) must be higher than the rest because they block only from the *trans* side. This is shown at the energy profile at the bottom. The Cs^+ -blocking site was determined previously⁶ and corresponds to $\delta = 0.37 \pm 0.01$ measured from the *cis* side; δ , when measured from the *trans* side is equal to $1 - \delta$ measured from the *cis* side.

We suggest two possible explanations for the different voltage dependences found for the two methonium blockers. As shown in Fig. 4, both molecules could possibly bind to the same site in the channel if hexamethonium blocks in a linear conformation with one charge inside the field and decamethonium in a bent conformation with both charges inside the field. In this case, the long-lived blocked states could be due to stabilization of the two charges of the molecules at the binding site. Alternatively, if both blockers act in bent conformations, the blocking site would be more external for hexamethonium than for decamethonium.

It is necessary to propose the unusual bent-over conformation for decamethonium (Fig. 4), with the two positively charged quaternary ammonium groups located in close proximity, both inside a channel structure, because of the following observations: (1) the channel seems to accommodate only one ion at a time¹²; (2) decamethonium blocks with an 'effective valence' of 1.2, indicating that more than one charge enters the field; (3) at least 18 different monovalent organic cations (decyltrimethylammonium among them) block the channel from the *trans* side with $z\delta = -0.62 \pm 0.03$ (R.C., unpublished results). Fortunately, decamethonium is a simple molecule, and it will be possible to synthesize several analogues with locked-in conformations which should reveal the molecular requirements for block with high affinity, long-lived blocked states and strong voltage dependence.

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Defective synthesis of HbE is due to reduced levels of β^E mRNA

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Haemoglobin E ($\alpha_2\beta_2^{26\text{Glu} \rightarrow \text{Lys}}$) is one of the commonest haemoglobin variants. There are an estimated 30 million carriers of the β^E gene in South-East Asia, where they comprise more than 50% of the population in some areas^{1,2}; however, the reasons for this high frequency have never been adequately explained. Homozygotes for HbE may be mildly anaemic, but they do not have any clinical disability^{3,4}. However, individuals heterozygous for both β^E and β thalassaemia (HbE/ β thalassaemia) have a severe clinical disorder which in some cases may approach that seen in homozygous β thalassaemia^{3,5,6} and which is by far the commonest form of symptomatic thalassaemia in the Indian subcontinent and South-East Asia. Haemoglobin E is the only common structural variant which interacts with β thalassaemia to produce such a severe disorder and the underlying mechanism of the interaction is not known. We have studied several homozygotes and heterozygotes for HbE and show here that the β^E chain is inefficiently synthesized and produces the phenotype of a mild form of β thalassaemia; hence, when inherited together with β thalassaemia it causes a marked β -chain deficit. Furthermore, the mechanism for the defective production of β^E chains seems to be a reduction of β^E mRNA, a most unexpected finding in a disorder caused by a single amino acid substitution and presumably by a single nucleotide change in the DNA of the β globin gene.

Five HbE homozygotes and nine heterozygotes were studied, all of South-East Asian origin. The presence of HbE was confirmed by peptide analysis in two cases and by a positive DCIP precipitation test⁷ in the remainder. The α gene status of all the cases was examined by restriction endonuclease mapping⁸ and, with the exception of a single α gene deletion in one of the HbE heterozygotes, normal α gene maps were obtained.

All the homozygotes showed a reduced haemoglobin level and microcytic, poorly haemoglobinized red cells (Fig. 1). The heterozygotes also had a reduced amount of haemoglobin per cell, as reported previously⁹, and the proportion of HbE was only 25-30% (Fig. 1), significantly less than the 35-45% normally observed in heterozygotes for stable β chain

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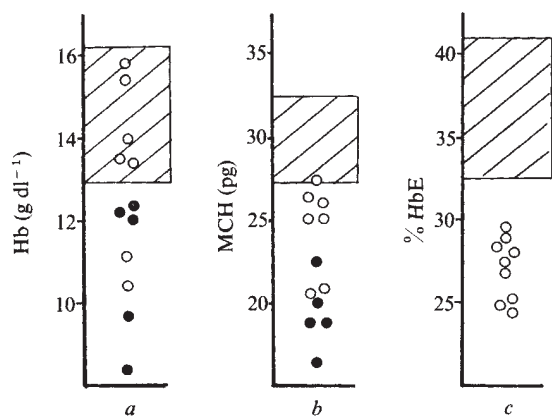


Fig. 1 Haemoglobin level (a), mean cell haemoglobin (b) and % HbE (c) in homozygous (●) and heterozygous (○) carriers of the β^E gene. The hatched area shows the normal range; in c this represents the % abnormal haemoglobin in a series of 16 heterozygotes for HbS, HbC and HbD, as measured in our laboratory.

variants¹⁰. Separation of peripheral blood erythrocytes according to age, by high-speed centrifugation, showed no change in the proportion of HbE between old and young cells, demonstrating that there is no preferential loss of HbE as the cells age.

The measurement of globin chain synthesis in peripheral blood reticulocytes of five HbE homozygotes showed a marked deficit of β^E chain synthesis (Fig. 2a), with α/β chain synthesis ratios similar to, or greater than, those normally observed in β thalassaemia heterozygotes. The synthesis of excess α chains in these cells was confirmed by gel filtration of the ³H-leucine-labelled reticulocyte lysates¹¹, where a pool of radioactive free α chains could be easily demonstrated. Furthermore, fine precipitates, typical of the free α chain precipitates found in β thalassaemia, were observed in the bone marrow normoblasts of two of these patients by electron microscopy (S. Wickramasinghe, personal communication).

Among the HbE heterozygotes, the $\alpha/\beta^A + \beta^E$ ratios ranged from 1.03 to 2.19 (Fig. 2a), which, with the exception of the individual who also had α thalassaemia, were always greater than those for the normal control incubation run in parallel. The mean β^A/β^E specific activity (c.p.m. per mg) ratio obtained after

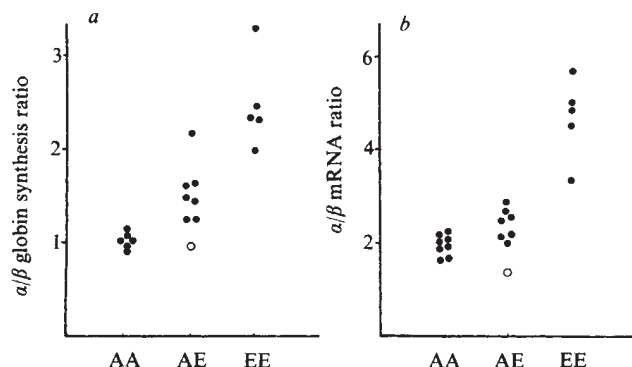


Fig. 2 a, Globin chain synthesis ratios in peripheral blood reticulocytes in normal (AA), heterozygous HbE (AE) and homozygous HbE (EE) individuals measured after 60 min incubation with ³H-leucine¹¹. b, Ratios of α/β globin RNA in the same individuals using the method described in ref. 14. The open symbols are the results from the individual shown by gene mapping to have one (out of four) α gene deleted.

60 min incubation was 1.24 ± 0.20 ; this provides further evidence that HbE is not an unstable haemoglobin, because if that were the case, such ratios would be less than 1.0.

The time course of globin chain synthesis was studied in three homozygotes and three heterozygotes (Fig. 3). Even at the earliest time point (2 min) there was no evidence that the specific activity of the β^E chains exceeded those of the α and β^A chains, and in the heterozygotes the β^A/β^E chain synthesis ratios remained constant throughout the incubation period. This argues against rapid destruction of the newly synthesized β^E chains.

Globin chain synthesis was also measured in bone marrow aspirates from three homozygotes and two heterozygotes and in each case the $\alpha/\text{non-}\alpha$ chain synthesis ratio was more balanced than in the corresponding peripheral blood sample (Fig. 4). This must in part reflect the proteolytic destruction of some of the excess α chains, as has been shown to occur in β thalassaemia heterozygotes^{12,13}. In the marrow of one of the heterozygotes the β^A/β^E synthesis ratio remained constant throughout a 60 min incubation, but in both heterozygotes this ratio was lower in the bone marrow (1.23, 0.91) than in the reticulocytes (1.51, 1.15) after a 60-min incubation.

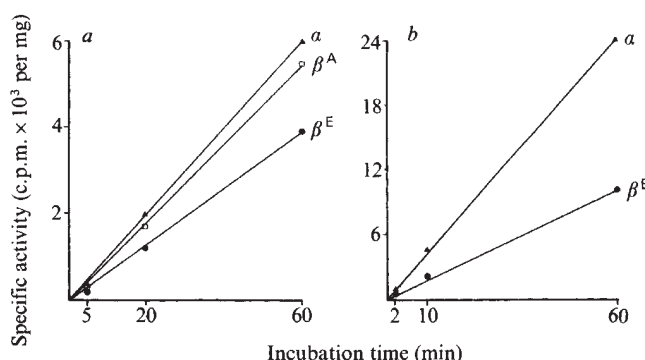


Fig. 3 a, The incorporation of ³H-leucine into the globin chains of a HbE heterozygote during a 60-min incubation of peripheral blood reticulocytes. b, A similar experiment with blood from a HbE homozygote.

These experiments produce clear evidence for the defective synthesis of β^E chains. To determine whether this occurs at, or before, the translational level, the amounts of α or β globin mRNA in the reticulocytes of these individuals were measured. We have reported previously¹⁴ that in carefully standardized conditions the α/β globin mRNA ratios fall into non-overlapping groups of normal individuals and those with various forms of α and β thalassaemia. The values obtained do not represent absolute mRNA values, but the results are valid for comparative purposes¹⁴. In the present study the α/β mRNA ratios in five HbE homozygotes (Fig. 2b) ranged from 3.29 to 5.81. This is two- to threefold higher than the range of 1.62–2.22 (mean 1.98) found in eight normal individuals studied at the same time, and similar to the values of 3.7 and 3.9 obtained in two β thalassaemia heterozygotes examined in this laboratory. Clearly, the deficit of β^E chain synthesis is reflected by a similar deficit of β^E mRNA production. Furthermore, in seven HbE heterozygotes the $\alpha/\beta^A + \beta^E$ mRNA ratios were significantly higher than in the normal controls (Fig. 2b).

These experiments demonstrate, therefore, that the low level of HbE is not primarily due to instability of the β^E chain¹⁵, or to incomplete association into tetramers¹⁶, as previously suggested, but results from deficient synthesis of β^E chains, as suggested by previous studies on isolated cases^{4,16,17}. The reduced synthesis of HbE (to approximately half normal levels) means that the β^E gene acts like a mild form of β thalassaemia and explains why, in the homozygous state, it produces a phenotype

similar to β thalassaemia minor, and why on interaction with a β thalassaemia gene, it produces a severe clinical disorder. The demonstration that deficient synthesis of the β^E chain results from lowered amounts of β^E mRNA is completely unexpected for a structural globin chain variant which results from a single amino acid substitution and hence presumably from a single nucleotide change. It is difficult to see how this single base change could alter globin gene transcription, but it is possible that its position at codon 26, close to the boundary of coding

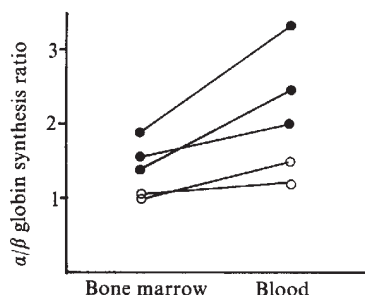


Fig. 4 Comparison of the α/β globin chain synthesis ratios in peripheral blood reticulocytes and bone marrow in three HbE homozygotes (●) and two HbE heterozygotes (○).

and intervening sequences after codon 30, might interfere with nuclear RNA processing. It is also possible that this particular nucleotide is involved in the secondary structure of the mRNA and that its substitution results in mRNA instability. The finding that the specific activity of β^E chains is lower than that of β^A chains in the reticulocyte incubations and the increase in the β^A/β^E globin synthesis ratios from bone marrow to reticulocytes would be consistent with this mechanism but this is very indirect evidence and could only be confirmed by measurements of bone marrow cytoplasmic mRNA ratios.

The remaining possibility is that the base substitution is not the cause of the decreased β^E chain synthesis but that there is a mild β thalassaemia mutation in *cis* to the β^E mutation. This could have arisen by sequential mutations in the same β globin gene, in either order, or by recombination between genes carrying the β^E and β thalassaemia lesions. If this were the case, the high frequency of the β^E gene in South-East Asia may be the result of selection for the β thalassaemia phenotype and the presence of the structural variant may be incidental.

Note added in proof: Reduced amounts of β^E mRNA have also been observed in patients with HbE/ β thalassaemia, as reported in abstract form by Berman *et al.*¹⁸

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Amino acid sequence homology between cholera toxin and *Escherichia coli* heat-labile toxin

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Cholera toxin (CT) and the *Escherichia coli* heat-labile toxin (LT) are functionally, structurally and immunologically similar enterotoxins. Both toxins cause the elevation of cyclic AMP levels in gut epithelial cells^{1,2} by catalysing the NAD-dependent ADP ribosylation of membrane proteins^{3–6}. Each toxin is composed of two dissimilar subunits^{7,8}. The A subunit has an enzymatic activity and is the adenylate cyclase-activating component of the enterotoxin^{4–11}. The B subunit recognizes membrane components and binds the holotoxin to the target cell juxtaposing the A subunit with its substrates^{9,12}. Binding studies and competition experiments indicate that the membrane receptors for cholera toxin B subunit (CT-B) and LT-B are similar but not identical¹³ (these studies were performed before LT was purified to homogeneity). The monosialosylganglioside GMI has been shown to be the receptor for the cholera toxin¹⁴, and it probably composes part of the receptor for LT. Gyles and Barnum¹⁵ first reported that LT and cholera toxin were immunologically related, and it has subsequently been shown that they share common antigenic determinants in both A and B subunits¹⁶. The primary structure of CT-B has been determined^{17,18}. We report here a comparison between the amino acid sequences of LT-B and CT-B. The nucleotide sequence of the LT-B cistron (*eltB*) was determined using a recombinant plasmid encoding LT⁹. Translation of this sequence revealed that LT-B and CT-B show significant amino acid sequence homology. In addition, several features of the *eltB* cistron were revealed by the sequence analysis.

Previously, we described the cloning of the LT genetic determinant⁹. Analysis of *in vitro* generated deletions in the LT gene showed that the gene was composed of two cistrons and established an approximate locus for both *eltA* (encoding LT-A) and *eltB* (encoding LT-B)⁹. The LT phenotypes encoded by various deletion plasmids indicated that *eltB* was located on a DNA fragment bounded by *HindIII* sites (Fig. 1). If *eltA* and *eltB* were adjacent cistrons, then the data further suggested that the N-terminus of LT-B would be coded by the smaller DNA fragment between the *HindIII* site and the *EcoRI* site. Figure 2 shows the nucleotide sequence around the *EcoRI* site. In most instances the nucleotide sequence of both strands was ascertained. The largest protein that could be encoded in this region was found to be 124 amino acids in length. The initiation codon for this protein was located 10 base pairs from the *EcoRI* site, but not in the small DNA fragment as initially speculated.

The nucleotide sequence encoding this protein was translated into an amino acid sequence which was compared with the sequence of CT-B (Fig. 3). The N-terminus of mature CT-B is threonine and the C-terminus is asparagine. The putative LT-B amino acid sequence also had asparagine as the C-terminal amino acid. If one compares the next 102 amino acid residues starting from the C-terminus, 80 of the LT-B residues are identical to those of CT-B. This result represents 79% homology. Of the 22 amino acid residues that differ, a single base change in the *eltB* codon could account for 20 of the changes. The other two nonhomologous amino acids would require a minimum of two base changes in the *eltB* codon to result in the homologous amino acid as found in CT-B. There are no regions in which the nonhomologous amino acids are concentrated, but rather they seem to be scattered throughout the primary sequence, except for a run of 23 residues (47–69) that are identical between the two proteins. A mature LT-B

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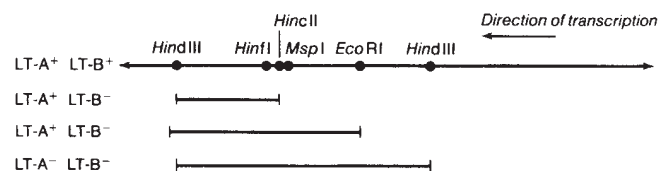


Fig. 1 Schematic diagram of the LT DNA region. Bars indicate plasmid DNA that was deleted⁹. The effect of each deletion on LT-A and LT-B expression is shown. Restriction enzyme recognition sequences used for DNA sequencing are also shown.

would have three more acidic amino acid residues than CT-B, whereas CT-B would have two more basic residues than does LT-B. CT-B has a compositional molecular weight of 11,590, whereas that of mature LT-B is 11,780, the difference corroborating our earlier reports that LT-B was slightly larger than CT-B (see note added in proof)^{8,9}.

The 21 additional amino acid residues encoded at the N-terminus of *eltB* are undoubtedly a leader sequence that directs the nascent protein through (into) the cell membrane(s) and is eventually cleaved from the molecule. This is suggested by: (1) A few prokaryotic leader sequences have been characterized and these sequences range from 20 to 23 residues in length²⁰; the LT-B leader sequence would be 21 amino acids in length. (2) Like prelipoprotein, the LT-B leader sequence would be cleaved after a glycine residue²¹. (3) The leader sequence of LT-B resembles the leader sequence of prelipoprotein in that both polypeptides have several charged amino acid residues at the N-terminus followed by a region, ~60%, composed of hydrophobic amino acids. (4) There is evidence that LT is either a periplasmic protein²² or an outer membrane-associated protein¹¹ and, therefore, it would be expected to possess a leader sequence.

One hundred and eighty nucleotides proximal to *eltB* have also been determined. None of these sequences has a Pribnow box-like structure which is characteristic of an RNA polymerase binding site. This result supports our earlier data which suggested that *eltA* and *eltB* are transcribed as a single polycistronic mRNA and that *eltA* was located next to the promoter⁹. There is a Shine-Dalgarno sequence²³ three nucleotides from the initiation codon of *eltB*. Five nucleotides in this region are complementary with five of the first 12 nucleotides at the 3' end of the 16S ribosomal RNA. The nucleotide adjacent to the initiation codon *eltB* is an A which has been reported to enhance ribosome

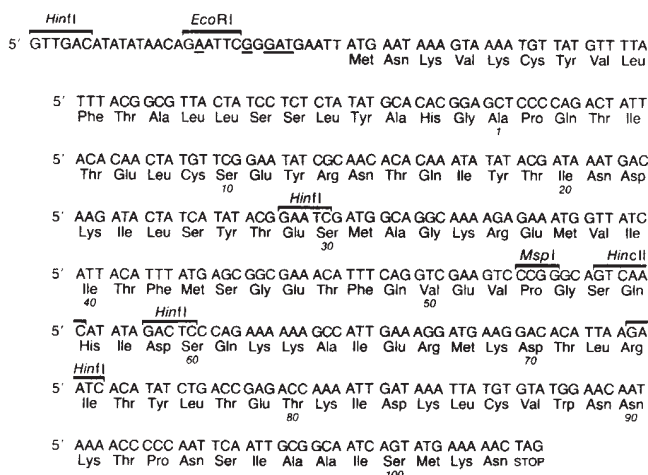


Fig. 2 Nucleotide sequence of *eltB*. The nucleotide sequence was determined using the chemical method of Maxam and Gilbert²⁴. DNA strands were radiolabelled by a kinase reaction or by end repair using DNA polymerase I Klenow fragment. The five underlined nucleotides are complementary with five of the first 12 nucleotides of the 16S ribosomal RNA. The amino acid labelled 1 is the proposed N-terminal amino acid residue of mature LT-B. Several restriction enzyme recognition sites are shown.

LT	CT	LT	CT	LT	CT	LT	CT	LT	CT	
Met		Ile	Ile	30	Ser	Ser	Ser	80	Thr	Ala
Asn		Thr	Thr		Met	Leu	Gln	Lys	Lys	
Lys		Glu	Asp		Ala	Ala	His	Ile	Val	
Val		Leu	Leu		Gly	Gly	Ile	Asp	Glu	
Lys		Cys	Cys		Lys	Lys	Asp	Lys	Lys	
Cys		10	Ser	Ala	Arg	Arg	60	Ser	Leu	
Tyr		Glu	Glu		Glu	Glu	Gln	Cys	Cys	
Val		Tyr	Tyr		Met	Met	Lys	Val	Val	
Leu		Arg	His		Val	Ala	Lys	Trp	Trp	
Phe		Asn	Asn		Ile	Ile	Ala	Asn	Asn	
Thr		Thr	Thr	40	Ile	Ile	Ile	90	Asn	Asn
Ala		Gln	Gln		Thr	Thr	Glu	Lys	Lys	
Leu		Ile	Ile		Phe	Phe	Arg	Thr	Thr	
Leu		Tyr	His		Met	Lys	Met	Pro	Pro	
Ser		Thr	Thr		Ser	Asn	Lys	Asn	His	
Ser		20	Ile	Leu	Gly	Gly	70	Asp	Ala	
Leu		Asn	Asn		Glu	Ala	Thr	Ile	Ile	
Tyr		Asp	Asn		Thr	Thr	Leu	Ala	Ala	
Ala		Lys	Lys		Phe	Phe	Arg	Ile	Ile	
His		Ile	Ile		Gln	Gln	Ile	Ile	Ile	
Gly		Leu	Phe	50	Val	Val	Thr	100	Ser	Ser
1	Ala	Thr	Ser		Glu	Glu	Tyr		Met	Met
	Pro	Gln	Tyr		Val	Val	Leu		Lys	Ala
	Gln	Gln	Thr		Pro	Pro	Thr		Asn	Asn
	Thr	Asn	Glu		Gly	Gly	Glu			

Fig. 3 Comparison of the amino acid sequence of LT-B and CT-B. The arrows indicate nonhomologous amino acids. Amino acids are numbered starting with the proposed N-terminal residue of mature LT-B.

binding²⁴. The presence of a ribosome binding site adjacent to *eltB* may explain the observation that LT-B is present in at least 5 M excess compared with LT-A, even though *eltA* is closer to the promoter⁹. This could be accounted for if the *eltB* ribosome binding site is more efficient than the *eltA* ribosome binding site and/or if the secondary structure of the mRNA enhances translation of *eltB* compared to *eltA*. Interestingly, no sequences characteristic of mRNA termination signals have been found in the DNA sequences distal to *eltB*. Preliminary sequencing information does suggest, however, that an additional protein may be encoded by the LT operon, but additional sequencing will be necessary to confirm or reject this conjecture.

Our sequencing information confirms the similarities between LT and CT. Knowledge of the nucleotide sequence of *eltB* should enable substitution of single amino acids by site-specific mutagenesis of a nucleotide in a codon and observation of the effect on protein function. The structural similarities shared by the adsorption proteins makes it tempting to speculate that immunity to LT-B might result in immunity to CT and neutralization of the toxin. In this regard, LT-B-producing *E. coli* might be potential live, oral vaccine candidates, both for LT-mediated diarrhoeal disease (in man and animals) and cholera.

We have successfully cloned *eltB* in such a way that it is being transcribed through the *lac* promoter and a protein is being made from this transcript that is structurally, functionally, and immunologically indiscernible from LT-B (manuscript in preparation). Using a strain harbouring this gene, we are pursuing the idea of an LT-B vaccine.

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Note added in proof: Clements *et al.*²⁵ have reported the first 20 amino acids of LT-B isolated from *E. coli* K-12 carrying one of our LT plasmids. These amino acids are identical with the first 20 amino acids of our predicted mature LT-B amino acid sequence.

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Freezing induced change in ligand orientation in oxycobalt-myoglobin

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Single crystals of oxycobalt-myoglobin were examined by electron paramagnetic resonance (EPR) spectroscopy at ambient and cryogenic temperatures. The principal values and eigenvectors of the *g*-tensor and the hyperfine coupling tensor were determined. The Co—O—O bond angle was estimated to be 125° at ambient temperature. The single crystal EPR data of oxycobalt myoglobin at 77 K showed two sets of the principal values for *g* and hyperfine coupling tensors and eigenvectors, indicating that the bound oxygen molecule takes two distinct orientations. The result has demonstrated for the first time that the well defined change in the molecular orientation is induced upon freezing the biological macromolecule.

Oxycobalt-myoglobin (oxyCoMb) was prepared by recombination of globin from sperm whale myoglobin and cobaltous protoporphyrin IX, followed by CM-cellulose column chromatography according to the method of Yonetani *et al.*¹. OxyCoMb was crystallized from ammonium sulphate-phosphate at pH 6.1 (refs 2, 3) in a space group *P*₂₁ with unit cell dimensions *a* = 64.51 Å, *b* = 31.03 Å, *c* = 34.82 Å, β = 105.84°, and a *Z* value of 2 (ref. 3). EPR measurements were performed with a Varian E-109 X-band spectrometer with 100 kHz field modulation, interfaced with a PDP 11/40 digital computer system for data acquisition and analysis. OxyCoMb crystals were mounted with a small amount of grease on a home-built two-circle Teflon goniometer⁴ and held in a Varian immersion Dewar flask for 77 K measurements or in a Varian variable temperature Dewar for higher temperature measurements. The crystal on the goniometer was manually rotated every 5° and/or 10° around its crystallographic axes with a reproducibility of $\pm 5^\circ$. The EPR data of the resonance positions versus the angle of rotation were computer-fitted to Schonland's equation⁵ to determine the angular dependence of *g*² and *A*² (the squares of the *g* values and hyperfine coupling-constants, respectively). The signs of the non-diagonal elements of *g*² and *A*² tensor were determined by measuring the angular dependence of *g* and *A* values around any direction in a single crystal with respect to its crystallographic axes. The principal values for these tensors and the eigenvectors with respect to the crystallographic axes, determined according to Schonland⁵, are summarized in Table 1.

At room temperature (300 K), oxyCoMb exhibits one unique set of *g* and *A* tensors, indicating that the paramagnetic centre or the metal-bound oxygen maintains a definitive molecular orientation relative to the crystallographic axes. The *g* and *A* tensors of oxyCoMb, however, do not share the same principal axis system with each other. The *A* tensors are principally determined by the 3d orbital of the cobalt ion. The EPR results

obtained at room temperature are compared with the X-ray structure of oxyCoMb, which was determined in an unfrozen state at -15°C (ref. 3), and schematically illustrated in Fig. 1. The ξ axis associated with $g_{\xi\xi} = 2.056$ does not lie along the O—O axis. Among the three principal axes of the *g*-tensor, the ζ axis associated with $g_{\zeta\zeta} = 2.003$ ($\approx g_e$ or the *g* value of free electron) appears to be the nearest direction to the O—O axis. From the directional cosines of *z* and ζ axes, the Co—O—O bond angle is calculated to be 125°, in good agreement with the results of the X-ray structural analysis³, in which the bound oxygen is shown to be oriented in the Pauling geometry with the Co—O—O bond angle of 129°. Note that the direction of the maximal *A* value, or *A*_{xx} = 18.12 G, is parallel to the imidazole plane (*C*₈—*C*₉) of the proximal histidine. Thus, the interaction between the *p* orbital of the imidazole ring of the proximal histidine and the 3d orbital of the cobalt ion, which has been previously proposed, cannot be verified from these results. The direction of *A*_{zz} is very close to the normal of the porphyrin plane within 5°, thus it is reasonable to assign *A*_{zz} to the direction of the Co—O₁ axis. However, the Co—O₁ bond is not strictly perpendicular to the porphyrin plane, but inclines approximately 8° from the normal of the porphyrin plane. On the other hand, the nearest axis to the direction of the O₁—O₂ axis is the ζ axis, which inclines about 20° from the direction of the O₁—O₂ axis. These angles are larger than our experimental error of 5°.

The unpaired electron, which is responsible for EPR in oxygen adducts of cobaltous chelates including cobaltous porphyrins has been generally assigned to the π^* orbital of the bound oxygen and the bound oxygen molecule has been oriented in such a way that its orbital overlaps maximally with the 3d orbitals of the cobalt ion in previous EPR studies of these compounds. The interaction between the π^* orbital of O₂ and the 3d_{z²} orbital of Co²⁺ (ref. 6) and the molecular orbital involving the π^* orbital of O₂ and the 3d_{yz} orbital of Co²⁺ (ref. 7) have been proposed. In these theoretical treatments, which assume the unpaired electron in the π^* orbital of O₂, the direction of the maximal *g* value ($g_{\xi\xi}$) should inevitably lie along the O—O axis⁷. Contrary to this assumption, our single-crystal EPR results at room temperature suggest that the direction of $g_{\xi\xi}$ ($\approx g_e$) does lie along the O—O axis. This suggests the possible contribution of the antibonding orbital of σ bond of O₂ to the metal-oxygen bond, although we could not exclude that of the π^* orbital. We believe that steric interaction of the distal residues such as E7 His, E11 Val, and CD1 Phe as well as the proximal histidine (F8 His) would contribute significantly to the stereochemistry of the Co—O₂ bond and should be

Table 1 The principal *A*^{Co} and *g* values and the directions in a single crystal

Complex	Temperature (K)	Principal values	†Angle to:		
			<i>a</i>	<i>b</i>	<i>c</i> [*]
O ₂ CoMb	300	<i>A</i> _{xx} = 18.12	85.4	± 50.2	40.1
		<i>A</i> _{yy} = 11.12	67.6	± 46.4	-51.9
		<i>A</i> _{zz} = 7.30	22.9	∓ 70.1	79.2
		<i>g</i> _{ξξ} = 2.056	56.3	± 48.3	60.0
		<i>g</i> _{ηη} = 2.011	66.1	∓ 42.2	57.6
		<i>g</i> _{ζζ} = 2.003	43.4	∓ 84.6	-47.1
O ₂ CoMb I	77	<i>A</i> _{xx} = 7.2	-62.3	∓ 33.1	73.3
		<i>A</i> _{yy} = 23.2	81.3	± 75.9	16.7
		<i>A</i> _{zz} = 11.6	29.9	∓ 60.1	-89.2
		<i>g</i> _{ξξ} = 2.08	59	± 82	32
		<i>g</i> _{ηη} = 2.03	45	∓ 50	-72
		<i>g</i> _{ζζ} = 1.98	60	± 41	-65
II	77	<i>A</i> _{xx} = 17.3	-76.2	∓ 22.0	73.2
		<i>A</i> _{yy} = 8.2	77.6	∓ 70.1	-23.7
		<i>A</i> _{zz} = 7.2	-18.8	± 81.0	-73.6
		<i>g</i> _{ξξ} = 2.085	-83.1	± 11.0	-81.6
		<i>g</i> _{ηη} = 2.008	-9.4	∓ 84.1	82.8
		<i>g</i> _{ζζ} = 1.983	83.8	± 80.8	11.1

†Upper sign refers to site A, lower to site B in unit cell²⁻⁴.

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incorporated in the theoretical calculation of the Co—O₂ bonding.

The single-crystal EPR data of oxyCoMb at 77 K are much more complex: two sets of the principal values for **g** and **A** tensors and eigenvectors are found, indicating that the bound oxygen molecule takes two distinct orientations (species I and II), confirming the previous observation of Chien and Dickinson⁸. However, these workers report different principal values and eigenvectors of **A** and **g** tensors from our own. The origin of these differences is not clear. However, there are two solutions for selecting the signs of the non-diagonal elements of **g**² or **A**² tensors, only one of which is correct. Figure 2 illustrates the Co—O—O bonding structure and the projections of the directions of **A**_{xx}, **A**_{yy}, and **g**_{zz} onto the porphyrin plane, together with those of proximal and distal residues, which are determined from our single-crystal EPR measurements at 77 K. If we assume that the direction of **A**_{zz} is fixed approximately to the normal of the porphyrin plane, the directions of the maximal **A** values for species I and II produce an angle of about 90° in the porphyrin plane. If the axis associated with its **g** values nearest to **g**_z is taken as the O—O axis, in accordance with the room temperature data, the Co—O—O bond angle becomes 93° (**g**_{zz} $\wedge$ **A**_{zz}) for species I and 163° (**g**_{zz} $\wedge$ **A**_{zz}) for species II, respectively. These two directions of the O—O axis nearly coincide with the direction of the imidazole plane (C₅—C₆) of the distal histidine. On the other hand, if the ξ axis (the direction of the maximal **g** value) is taken to be the O—O axis, the Co—O—O bond angle becomes 111° for species I and 108° for species II, and each ξ axis is oriented almost in the direction of its maximal **A**-value, respectively. However, it cannot be determined which axis should be the O—O axis in the 77 K EPR conditions. Whichever one is taken as the O—O axis, the direction of the O—O axis at 77 K is definitely different from that observed at room temperature.

In a previous single-crystal EPR study at 77 K (ref. 8), it was concluded that the bound oxygen molecule in oxyCoMb takes two types of orientation (species I and II) which are more or less in the Griffith parallel geometry. However, we find that the two orientations of oxygen in oxyCoMb at 77 K are best described

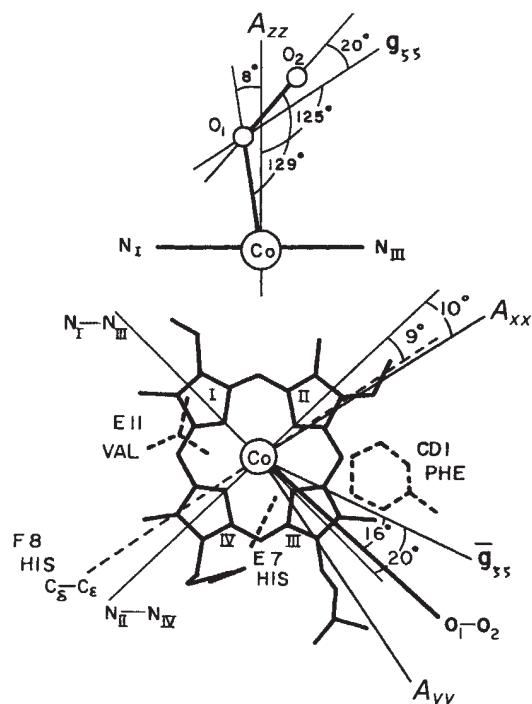


Fig. 1 The bonding structure of Co—O₂ and the directions of **g** and **A** tensor (room temperature): Co—O—O bond angle and the directions of **A**_{zz} and **g**_{zz} are shown (upper). The projections of **A**_{xx}, **A**_{yy}, **g**_{zz}, and O—O axis onto the porphyrin plane are shown (lower), from the proximal-side view. The projections of the distal residues are shown by dotted lines.

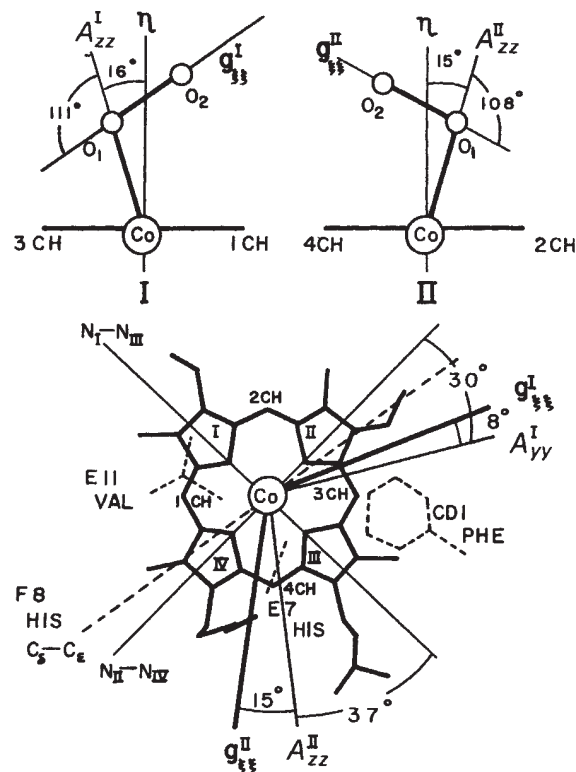


Fig. 2 The bonding structure of Co—O₂ (77 K); the directions of the maximal **A**-values for species I (**A**_{yy}) and for species II (**A**_{xx}) are shown (lower). ξ -axis is assumed to be fixed on the O—O axis. The two species (I and II) are oriented about 90° to each other in the crystal (upper).

by Pauling bent geometry, neither of which is identical to its Pauling orientation at room temperature (see Figs 1 and 2). EPR measurements at different temperatures indicated that the transition between one potential-well at room temperature and two potential-wells at 77 K for the oxygen binding in CoMb is a reversible process and takes place at/near the freezing point of crystals.

Adverse effects of solvent freezing on biological compounds are well recognized but only limited data are currently available. Examples are abrupt changes in the paramagnetic susceptibility in haemoglobin near freezing point⁹ and the loss of the ordered intermolecular arrangement in crystals of cytochrome *c* peroxidase on freezing¹⁰. The present single-crystal EPR study shows for the first time that a well defined change in the molecular orientation is induced by freezing of a biological macromolecule. Since magnetic and spectroscopic measurements of biological macromolecules, particularly metalloproteins, are by necessity carried out in a wide range of ambient and cryogenic temperatures, it is important to recognize that the analyses of experimental data and theoretical interpretation of such data must take into account the possible adverse effects of freezing, as demonstrated here.

We thank Dr G. Petsko for the coordinates of the X-ray structure of oxyCoMb and Drs G. Petsko, P. Douzou, and T. Inubushi for stimulating discussions. This work was supported by research grants from the National Heart, Lung, and Blood Institute (HL 14508) and the NSF (PCM79-22841).

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CHRISTMAS BOOKS SUPPLEMENT

From Dan to Beersheba, and beyond

P.J. Parr

THERE was a time, in the late nineteenth and early twentieth centuries, when a desire to illuminate, explain and even to vindicate the Bible provided the chief motive force behind much of the archaeological exploration of the Near East — of Mesopotamia and Egypt, and of Palestine itself. Archaeology has changed in recent years, however, both in its goals and its methods, and its practitioners in the Holy Land today are likely to be as concerned with such problems as the diet of Neolithic man or the mechanisms of Bronze Age trade as they are with the date of the Exodus, the design of Solomon's temple or the location of the tomb of Christ. In academic circles Biblical archaeology has certainly lost much of its former prestige; which is, on the whole, not a bad thing. Nevertheless, it remains probably the most popular branch of the subject amongst the educated lay public, for the simple reason that Palestine — as Moshe Pearlman admirably puts it in his book *Digging up the Bible* (Morrow/Weidenfeld & Nicolson, \$19.95/£8.95) — “is the only region of antiquity of which there is a written historical record that has been familiar to so many people in every generation for so long”. The writer of a book catering for this interest has a duty to be readable, accurate and reliable, and to add something new to the already vast literature available, and it is by these criteria that all of the three new books reviewed here should be judged.

Pearlman's volume is certainly the least satisfactory, despite the fact that it is well-written and produced, with many pertinent and attractive illustrations and a lively text. His re-telling of the “stories behind the great archaeological discoveries of the Holy Land” is substantially accurate, though they have all been told before. It is a pity that, as the account nears the present day, there is a definite over-emphasis on

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Erosion features in the Sinai

Israeli research at the expense of that carried out by foreign scholars; for example, the important work of Kathleen Kenyon at Jerusalem just before the Six Day War receives scant mention, while the excavations of Roland de Vaux (one of France's most outstanding Biblical archaeologists) at Qumran, where the Dead Sea Scrolls were written, are not referred to at all, although the Scrolls themselves are given a dozen pages of text. The book's chief fault lies in the dubious nature of some of its archaeological statements, however. Thus (at random) the Hyksos are no longer seen as a “northern people” by most scholars (p.146); the date of the Exodus has not been proved just by the discovery of a few pieces of Mycenaean pottery at Hazor (pp.71–72) — it has, in fact, not yet been satisfactorily established that there really was an Exodus; the date of the Neolithic tower at Jericho has been known for over a decade to be at least a thousand years earlier than the date of 6850 BC quoted on p.141. Even a popular book has to be less misleading and simplistic than this.

Father Murphy O'Connor's *The Holy Land: An Archaeological Guide from Early Times* (Oxford University Press; hbk

£8.50, \$15; pbk £3.95, \$5.95) is much more reliable, though it too has its faults. Almost everything written on the dust jacket is true: it is “concise, readable and wittily erudite . . . its scope is wide . . . it gives clear directions [and has] numerous detailed plans”. It differs from — and adds to — most comparable books by giving a prominent place to many of the less well known and accessible sites and monuments, especially of the Byzantine and Crusader periods, with which the author — who is Professor of New Testament at the Ecole Biblique in Jerusalem — is clearly most familiar. It is, conversely, perhaps a little thin on some of the pre-Israelite remains (the important Canaanite temples of Area H at Hazor, for example), and the brief discussion in the opening pages of the early history of the country is really much too short to be useful to anyone, and includes some statements (for instance the date of the Neolithic Revolution; the date and nature of Abraham's journey from Mesopotamia) which are as simplistic and inaccurate as some of Pearlman's. But this is the only flaw in an otherwise excellent guide, which should be in the luggage of every visitor to the Holy Land.

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Simon McBride

The third volume, *Sinai* (Kümmerly and Frey, Berne, SF88), edited by Beno Rothenberg, is remarkable for achieving a very successful synthesis of attractive coffee-table format and serious academic text between the same two covers. Its hundred-odd colour photographs, by Helfried Weyer, are outstandingly beautiful, and the only danger is that they will distract the reader from the text. This would be a pity. It consists of a number of concise but scholarly chapters not only on the archaeology and history (ancient and modern) of the peninsula, but also on the environmental background (geology,

Sinai is available in the UK from The Museum Bookshop, 36 Great Russell St, London WC1 3PP, price £20, and in the US from J.J. Binns, 6919 Radnor Rd, Bethesda, Maryland.

mineral resources, flora, fauna) essential for an appreciation of that history. The archaeological chapters are based mainly on the fieldwork of Rothenberg himself, and present some of the results for the first time in English. Rothenberg does not avoid technicalities and sometimes indulges in jargon; but the terms are usually explained, the arguments logically presented and the accompanying plans and diagrams attractively informative — all in the best traditions of popularization. These archaeological discoveries in *Sinai* are mostly related to periods long pre-dating the Israelites, and have little direct bearing on Biblical archaeology, in its narrow sense. But the book provides a vivid and reliable demonstration of the sorts of problems and categories of evidence now engaging the attention of archaeologists in Palestine, and as such is to be warmly recommended. □

reproduced are fuzzy and faint, and are plainly failures which a critical photographer normally consigns to the waste-paper basket. They do not captivate and entrance us, as the author surmises. For her they represent “a dream world of symbols and queer relationships . . . that naively ask questions pertaining to life and death, time and motion”. The author slips quite easily from science to poetic flights, a nice feature but we have to come back to the ground. The poor reproduction of the photographs is due to a cardinal mistake in this American production in printing them on text instead of art paper. Neither in colour nor in contrast do the sepia reproductions approach the quality of the originals. Only those few examples printed in red, purple and primrose — colours imparted to the calotypes by use of different fixing salts — fulfil this expectation, and they are beautiful.

In any new edition I would recommend the correction of a few statements; I will mention just three. Talbot's discovery of the latent image was a vital element in his calotype process. Yet Daguerre had made the same discovery five years earlier in his. It is therefore wrong to use the term “photography” which covers both methods. Talbot's wife Constance was not the first woman photographer. Madame Giroux, wife of the manufacturer of the official Daguerre camera, in 1839–1840 supplied each outfit with a sample daguerreotype taken by herself. *The Pencil of Nature* was published in six parts over a period of two years, the last appearing in April 1846 (not 1845).

The history of photography has been enriched by two recent biographies of one of the inventors of the process. Both of them are valuable additions to the literature, but neither is objective enough to be considered definitive. Hero worship is a poor partner of truth. □

Negative and positive of Fox Talbot

Helmut Gernsheim

Fox Talbot and the Invention of Photography. By Gail Buckland. Pp.216. (Scolar/David Godine: 1980.) £20, \$50.

WILLIAM Henry Fox Talbot FRS, inventor of the negative/positive method of photography on which modern photography is based, patentee of halftone photo-engraving, of electric flash photography and a number of other important contributions to the development of photography, was a brilliant scholar in several fields of science. Yet an unfortunate mania for pursuing patents, for commercial enterprises and for law suits earned him more animosity than cash the impoverished owner of the ancient abbey and village of Lacock needed to rebuild the family fortunes.

In his belief that every photographic process with the exception of the daguerreotype was only a variation of his own, Fox Talbot effectively prevented important improvements, freely published by others, from becoming common property in England, thus severely impeding the progress of photography. However, his claim to Frederick Scott Archer's collodion process on glass, which was about to oust his own method on paper, outraged English photographers, fellow scientists and editors of journals and they combined to defend photography from further interference; Talbot lost the case in December 1854. With the new-won freedom to compete with other countries, a golden age of invention and creativity set in giving Britain a leading position with France in the latter half of the nineteenth century. As neither Daguerre's nor Talbot's patents included Scotland the position there was quite different. The finest calotype portraits and some of the

best waxed-paper photographs in existence were produced in Edinburgh in the 1840s and early 1850s.

Probably out of a false respect for her hero, little of this strangulation of English photography in its early formative period (1841–1854) emerges in Mrs Buckland's book. Similarly, H. J. P. Arnold, in his biography of Talbot (Hutchinson, 1977) which preceded hers by three years, tried hard to belittle the strong contemporary evidence of Talbot's machinations. Anyone interested in a rather different view of Talbot can find it in my *History of Photography* (Oxford University Press, 2nd Edn 1969) and in R. Derek Wood's detailed study of Talbot's law suits (*Annals of Science* 1971 and 1975).

The 230 photographs partly integrated with Mrs Buckland's text, and partly added to it in album fashion, plus the many interesting excerpts from letters and journals, make her monograph particularly valuable. Yet many of the calotypes

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“The Open Door”, March 1843. The picture that made Talbot “certain that he could give form to his creative instincts . . .”.

More variations on a biological theme

Ashley Montagu

The Panda's Thumb. By Stephen Jay Gould. Pp.343. (W.W. Norton: 1980.) \$11.95. To be published in the UK in spring 1981, £6.95.

RECENTLY a young student of mine, having ably presented a seminar on Stephen Jay Gould's admirable book, *Ontogeny and Phylogeny* (Harvard University Press, 1977), asked me what Gould's specialty was. Only one answer was possible: versatility. Gould is a professor at Harvard and lectures on geology and palaeontology; he also teaches courses in biology and the history of science, and writes on physical and biological anthropology, growth and development, evolutionary theory, human nature, intelligence testing and sociobiology, to name but a few areas of his competence. In addition he is a highly regarded reviewer in scientific and literary journals and magazines. To cap it all, he writes a monthly column, "This View of Life", in the magazine *Natural History*.

It is from the column that this anthology, lightly edited, has been put together. Gould's earlier collection of essays from the same source, *Ever Since Darwin* (W.W. Norton), appeared in 1977. That volume immediately established him as perhaps the best of our natural history essayists. As a friend remarked the other day, "Whatever he writes is a gift".

In the prologue to the 31 pieces which make up this new volume, Gould expresses the hope that he has avoided that incubus of essay collections: diffuse incoherence. Neither author nor reader need have any qualms on that or any other score: the essays are consistently coherent and follow naturally, one upon the other, as if they were chapters on a single theme. There is a continuity which runs through this eminently readable book like a red thread, giving it a unity behind which an unusual mind is at work trying to figure out, among other things, how multicellular creatures regulate the timing involved in the complex orchestration of their embryonic growth, in the hope that developmental biology might one day unite molecular genetics with natural history to form a unified science of life. In one way or another these essays are mostly directed toward illuminating the routes to be taken to solve that problem.

The book plays variations upon this theme most delightfully, for in addition to being the best-informed of writers, Gould is also one of the most elegant. He is unfailingly interesting, for he has the rare gift of communicating the excitement he feels about whatever his fertile brain encounters. The reader comes away enriched and entertained at the same time. From the first essay, from which the book takes its title, in which the author discusses

the manner and the method of evolution, to the last, in which he shows, by way of the chambered nautilus, how the palaeontologist has come to the rescue of the geophysicist and mathematician in arriving at a sound estimate of the rotational slowing of our planet, Gould holds the general principle of evolution steadily in view.

In an essay on Darwin and Wallace, Gould shows how the latter, one of the most remarkable and underestimated of thinkers, ultimately became an intellectual victim of his own hyperselectionism, in arguing that the excessive complexity of the human brain could not have been produced by selection. In another essay, "Darwin's Middle Road", the author writes revealingly of the fibs that the great man told about his insights. Here it is good to see Gould recognizing what few ever do in discussing the historical background of the Wallace-Darwin theory, namely, the role played by sociopolitical ideas, a fact clarified by Patrick Geddes in the late

nineteenth century and by Charles Sanders Peirce almost as long ago.

In this essay Gould errs in saying that what was presented at the famous Linnean Society meeting of 1858 was "a joint paper". Though this is often stated, the truth is that what was presented, in the absence of both men, was an extract communicated in 1844 to Lyell and a letter to Asa Gray of 1857 (both by Darwin) and at the same time, but separately, Wallace's 1858 essay "On The Tendency of Variations to Depart from the Original Type". The communications were presented *together*. There was no joint paper as such.

But Gould rarely nods. On the few occasions on which he does he challenges one furiously to think. His book is a treasure. □

Ashley Montagu is a biological and cultural anthropologist. He is editor of the recently published Sociobiology Examined (Oxford University Press).

Contrary natures

Richard Mabey

THE ideal countryside of the book-packager is all things to all possible buyers: visible, walkable, edible, amenable to identification and to technicolour display, wonderfully awesome without ever being fundamentally mysterious. When Felix Gluck Press put together *Nature Through the Seasons* and *Nature Day and Night* for Penguin/Viking (now issued as paperbacks at £1.95/\$4.95 and £2.50/\$7.95 respectively) they took this principle to its depressingly logical conclusion and interweaved quite separate contributions by a writer, an artist and a scientist — the days being long past, presumably, when one person could be expected to be all three. The series of cameos in each book begins with an imaginative scene setter ("A Rocky Coast by Day", for example), continues with a "science text" ("Tides and Internal Clocks"), and is rounded off by one of those breathless habitat collages which look as if they are depicting local assembly points for the Ark.

Max Hooper's science notes are, as you would expect, lively and entertaining; David Goddard's set-pieces — all sharp edges and precise little tessellations of colour — are meant, I suppose, to be some kind of half-way stage between the scientific and poetic visions, but end up looking as stiffly unnatural as paintings-by-numbers. Neither seem to grow in any convincing way out of Richard Adams's wonderfully evocative introductory essays. For anyone who finds his fiction a little

indigestible these are a revelation. He meanders through his favourite landscapes, sniffing, listening, exulting, reflecting, but without being intrusive in any way, and succeeds in capturing the exact flavour of *moments* in nature, from the small hint of spring in the first brimstone butterfly, to the flowing away of the whole year in a leafless winter wood.

The ingredients which have been sandwiched together in Hodder and Stoughton's series *The Natural History of Britain and Northern Europe* are a lightning guide to the ecology of each habitat and a European field guide. The latest volume by Brian Whitton, *Rivers, Lakes and Marshes* (£5.50), ends up being rather unsatisfying on both counts. It was surely a mistake to try and produce a digest of the aquatic flora and fauna of half a continent, couched in scientific language yet marketed for tourists. What would your botanically-inclined riverside picnicker make, I wonder, of a branched bur-reed whose description reads "Lvs erect, <15mm wide, keeled, triangular in section, ♂ and ♀ fls in separate, spherical heads; in branched inflorescence, ♂ above, ♀ below"? A bee-line for the sandwiches rather than the glossary, I would imagine.

At least *Nature Detective* by Hugh Falkus (Penguin, £2.95) takes the view that there is more to the study of nature than the naming of parts, and presents an entertaining guide to forensic field biology. The countryside is littered with physical clues

about animal encounters: tracks, scratchings, suspicious mounds, discarded skins, decapitated corpses, each one with its distinctive "MO" as they call it in Criminal Records. Again, it is a rather narrowly materialistic view of the natural world, but at least the tone of the writing — and of the captions in particular — encourages you to go sleuthing with a light heart. The photographs, mostly by Niko Tinbergen, are superb, and take this book beyond the purely anecdotal. One, of the heraldic print of a raven's wings in deep snow, suggests an ancient survival myth way outside admissible evidence.

One needs to be something of a nature detective to work out what on Earth is going on in the anonymous *The Twelve Months of the Year* (David and Charles; hbk £5.95, \$16.95; pbk £3.95, \$11.50) so scrappy, inaccurate and disorganized are its contents. But clues to the conspiracy (and the motivation) are plain enough: a photograph of a red-backed shrike with young (in the February section!) mislabelled as a great grey shrike; common mouse-ear equated with chickweed — rather alarmingly, since a few pages later you are encouraged to eat it; those habitat collages again, with crows cohabiting with cranes, black woodpeckers in the beechwoods and hamsters in the corn. This is a world we have never encountered naturally in Britain, and you do not need to check in the picture credits to realize that this was originally a Dutch volume which has been hurriedly adapted, entirely unexplained, for a British audience. It is as cynical an exercise in book-making as I have ever seen, and I feel rather sorry for the British author who has translated or added to parts of the text (unacknowledged, even though he or she writes in the first person) as these fragments are really rather good.

Gordon Benningfield has provided (and signed) both text and pictures for *Benningfield's Countryside* (Allen Lane/Viking, £7.95/\$19.95). His paintings are as accomplished and idyllic as usual.

What is the surprise in this book is the quality of his prose, which has a mixture of unaffected strength and personal vision that is now sadly rare in English country literature. He writes uncompromisingly about the devastation wrought to his childhood countryside, about the forces that draw him to painting, and best of all about the business of putting a picture together. I must confess that I like his big soft, pastoral landscapes least of all his work, but his notes on why they were composed just so at least made me look at them with a new curiosity:

I have also put a glimpse of deer into my large painting of a typical piece of Ashridge [in Hertfordshire] at bluebell time, in May . . . The deer have just seen me and are about to move off; in a moment, they will have disappeared altogether. I wanted to convey the feeling that wildlife is not at all obvious in the countryside, that it is just a piece of luck that it has appeared at this moment.

Along with his prose, I think Benningfield's strengths lie in his detailed portraits, quite unidealized, of butterflies and gates and mucky field corners. It would be exciting, then, to see some of the outrage he expresses in his writing worked out in his painting.

Compartmentalization of the ways we look at and talk about the natural world is now dangerously endemic. Gordon

Benningfield is a man of sufficient popular appeal and broad-based skills to be able to make the kind of coherent personal statements that are normally anathema to contemporary publishers. □

Richard Mabey is a writer specializing in conservation and countryside matters. His most recent book is The Flowering of Britain, published by Hutchinson.

In the wake of the *Beagle*

Arthur Bourne

The Yachtsman's Naturalist. By Maldwin Drummond and Paul Rodhouse. Pp.270. (Angus & Robertson: 1980.) £8.95.

NOSING about the coastlines of western and north-western Europe covered by this book is one of the most rewarding occupations open to the yachtsman. These rewards are greatly increased if the navigator combines his art with an understanding of and a curiosity about the natural world around and beneath him. If his curiosity extends to exploring that world, then it requires only a small push to hook him into doing some real science.

The push can come in many guises, most often through the infectious enthusiasm of a friend or a book. There used to be an excellent book but it is now long out of print and most of us have had to rely on the enthusiastic friend. So there is a need for a guide designed especially for the amateur marine biologist and oceanographer and the present volume is intended to fill that need. The question is does it do so?

In Part one, which consists of three chapters, the authors describe the ecology of the coastal zone, from the land, down the shore to the water column itself. This part of the book is excellent and one could not wish for a better introduction to the subject. Part two, covering seamanship and equipment, has only two chapters and is more condensed. It is less satisfactory and gives the feeling that the authors were in a hurry and used scissors and paste in an attempt to finish quickly.

The chapter on seamanship and long-shoremanship begins with an interesting and informative description of the sort of seamanship required of the yachtsman if he is to navigate and lie safely close in to the shore — necessary if he is to observe the natural scene to best advantage. Such matters as the most effective way of anchoring, types of anchors and their weights, lengths of warp are dealt with in some detail, including the all-important — and often neglected — subject of drying out. Unfortunately little else! The equipment needed for the yachtsman/naturalist (now, by the way, called the sailor/naturalist), including the yacht

itself, comes in the final chapter. My own preference would have been to have placed choosing a yacht right at the start of Part two and I would have liked to have seen some more detail. The authors give only two pages to what is not only the most expensive piece of equipment but the most important. The rest of the equipment and its use is covered reasonably comprehensively, though the amount of detail varies and reflects, probably, the interests of the authors.

A feature of the book is the colour plates, illustrating animals and plants of the coast and sea, which are slotted into the book in six groups of two. The names of the organisms are provided on the backs of the plates and a numbered key aids rapid identification. The same key is used in the text where the organisms are mentioned. This arrangement works well. The plates themselves are adequate; one can recognize the organisms and perhaps that is all one requires. They do make the book colourful but I cannot agree with the authors that the plates make the book. They do not. What makes the book is the writing and the obvious enthusiasm of the writers. There are also large numbers of line drawings but these are poor except for those showing equipment and boats where the artist is particularly good at portraying yachts in the transparent shorescapes.

These are only small criticisms and perhaps we should go back to the question as to whether the book fills the need? Yes and no. As a personal preference I would like to have seen Part two as well developed as Part one, but, overall, the authors have provided a sound, useful and stimulating volume. I suppose one should ask of this sort of book whether it works, given its limitations, often dictated by constraints of size and costs. Would a yachtsman with this book be able to increase his knowledge of the natural scene about him? I believe the answer must be an unqualified yes. I hope it will find a place on the shelves of yachtsmen and naturalists alike. □

Arthur Bourne has a special interest in coastal zone ecology and has been a member of many shipborne expeditions from the tropics to the higher latitudes.

Rare gifts of botanical illustration

Peter D. Moore

THE art of botanical illustration has presented a great challenge to many who have been fascinated by the structure and colour of flowers, and who have sought to represent these in two dimensions. Science is itself an attempt to describe with precision the world in which we live, and the graphic arts have proved a valuable consort to the physical sciences and mathematics in achieving this end. In no area of science is this more true than in botany, where much early, observational work was assisted by the skills of artistic illustration. The three books reviewed here are splendid examples of the contribution which art makes to the understanding, appreciation and enjoyment of plants and, indeed, the contribution made by the plant to art.

The life and work of Marianne North, set out in *A Vision of Eden* (Holt, Rinehart and Winston/Webb and Bower, \$22.50/£8.95), is a prime example of the mixing of art and science. It is the autobiography of an unmarried Victorian lady who, at the age of 40 and on the death of her father, took to a life of travel, and recorded in paintings the vegetation of the areas she visited. It is a story of amazing courage, for the hazards of travel in the 1870s were quite formidable, but she tackled both these and her art with devotion and fortitude. The result was a large collection of botanical illustrations from many areas of the world and an extensive selection of these is presented in this book.



Marianne North, 1886

An interesting aspect of Marianne North's work is the varied way in which she presented her botanical subjects. Some illustrations are entirely devoted to a single plant, in which close detail of structure is represented. Some take the form of still-life groups in delicate arrangements. Other plants are shown in natural settings, with birds and butterflies and other insects in

attendance. But, to my mind, her most artistically appealing works are those in which the plant becomes the foreground to a landscape. Her paintings from northern India, for example, together with her vivid descriptions of the dust, the heat and the inevitable sickness, rival the works of Forster and Masters in conveying the unique atmosphere of that part of the world. As a book of travel stories from a century ago, this book is particularly valuable, for it provides us with botanically detailed descriptions of many areas which, like the Himalayas, are now greatly changed under the impact of increasing human exploitation.

Among our modern painters of flowers, the name of Marjorie Blamey is outstanding. In her latest book, *Flowers of the Countryside*, produced in cooperation with Philip Blamey (Collins/Morrow, £8.95/\$25) she has assumed the role of author as well as illustrator. The text is something of a botanical scrapbook, parts autobiographical, parts historical and parts anecdotal. It is in no way a botanical textbook and it is unclear what purpose it is intended to serve. A large portion of the book consists of a survey of the major British plant families, of which selected species are illustrated and described; yet this cannot be regarded as a field guide. The paintings are both accurate and pleasing, and I am sure that many who buy this book will be motivated by an admiration of these pictures, which are generally well produced and whose colours are more realistic and less garish than some recent efforts by Collins to reproduce Marjorie Blamey's work.

Another collection of botanical jottings which is rather more tightly packed with interesting items of science and folk-lore, is Richard Mabey's book, *The Flowering of Britain* (Hutchinson, £9.95). The style is informal and will appeal to those who find information easier to accept and assimilate when presented in a chatty manner. No scientist should object to this whilst accuracy is retained. The historical and other tales in this book make very good reading and deserve to be read widely.

On the art side, Richard Mabey has found a very able associate in Tony Evans, whose colour photographs are superior to any I have previously seen in print. The atmosphere of his photographs is brilliantly conveyed by the careful setting of the plant subject in its surroundings. The unity of plant and habitat which results is reminiscent of the paintings of Marianne North and is equally striking. The camera may have replaced the brush in the equipment of the latter-day botanist, but the art of composition is still a rare gift: Tony Evans, in my opinion, has provided a new dimension to the art of botanical illustration.



Lords and ladies, by Marjorie Blamey

From *Flowers of the Countryside*, by Marjorie and Philip Blamey

Peter D. Moore is Senior Lecturer in the Department of Plant Sciences, King's College, London. His most recent book is *The Mitchell Beazley Pocket Guide to Wild Flowers*, published in October of this year.

Ornithology in art and art in ornithology

T. R. Halliday

IT IS one of the finer traditions of publishing that books on natural history have been illustrated by artists, some of whom have gained recognition within a wider artistic world. Three new books are richly endowed with the work of three of Britain's finest bird painters: Peter Scott, Charles Tunnicliffe and George Lodge. Peter Scott's autobiographical *Observations of Wildlife* (Phaidon/Cornell, £7.95/\$19.95) contains four chapters which deal with his major lifetime preoccupations as a painter and naturalist, as creator of the Wildfowl Trust, as a leading figure in conservation, and as a traveller and ambassador for the conservationist cause. Peter Scott himself draws attention to a feature of his work that some critics have deplored, that many of his paintings are just variations on the theme of a flight of birds against a dramatic sky. He attributes this to the demands of his market which have led him to paint to a "formula which guarantees sales but limits invention and innovation". Happily, this book bears testament to an artist of very diverse talents. The sketches from his diaries, which include studies of insects and fish as well as birds, are unexpected and exquisite. Peter Scott's text reminds us of the many important ways in which he has contributed to a greater understanding of nature and of conservation in particular, and it is understandable that his art has not been as varied and innovative as it might have been.

In contrast, Charles Tunnicliffe was an artist who devoted his entire life to his craft and Ian Niall's affectionate biography *Portrait of a Country Artist* (Gollancz, £10) contains vivid evidence of his many and varied skills. Here we find a rich variety



Charles Tunnicliffe at work, January 1952

of both subject matter and technique. Some of his oil paintings on unprimed cloth have a distinctly oriental flavour, while his woodcuts and etchings remind one of Durer. Sadly, Tunnicliffe's paintings sometimes seem over-posed and excessively pretty and have lost the life and vigour that he captured so brilliantly in his sketches. The basis of his work was a meticulous attention to detail which is best seen in his beautiful post-mortem studies of birds. The visual feast that his book provides is admirably complemented by Ian Niall's informative and interesting portrait of an exceptionally talented and self-effacing man.

A Season of Birds (Weidenfeld & Nicolson/A&W, £7.50/\$14.95) is a less successful book. The text consists of a diary kept by Jim Vincent in 1911 on the

wildlife of Hickling Broad, Norfolk, for which Edwin Montague commissioned illustrations from G.E. Lodge. The text is likely to be of interest only to the most ardent bird-watcher as much of it consists simply of a list of species seen on each day. Lodge's watercolours are reminiscent of Thorburn's work but are not nearly as successful at capturing the essential characters of their subjects. Lodge's reputation is mainly based on his painting of birds of prey and it is clear from this book that he was not nearly as good at portraying other kinds of bird. □

T. R. Halliday is a Lecturer in Biology at the Open University and an author and illustrator of a number of books on natural history. His most recent volume is Sexual Strategy, published by Oxford University Press in October of this year.

There was a young artist called Lear . . .

M. A. Ogilvie

Edward Lear's Birds. By Susan Hyman. Pp. 96. (Weidenfeld & Nicolson/Morrow: 1980.) £18.50, \$37.95.

THERE was an old man who said 'Hush!
I perceive a young bird in this bush!'
When they said - 'Is it small?' He replied -
'Not at all!
It is four times as big as the bush!'

Who else could have written that but Edward Lear? He did not invent limericks, but he certainly wrote some of the best there are, and illustrated them with his inimitable line drawings. To these add his nonsense verses ("The Owl and the Pussycat", "The Dong with the Luminous Nose"), and one probably has the sum of most people's knowledge of him. This handsomely produced and lavishly illustrated book demonstrates just how restrict-

ed that knowledge is. The limericks and nonsense verses were not published until Lear was nearly 60, but by that time he had proved himself firstly as one of the finest natural history artists of all time, and then, abandoning this field before he was 30 because of failing eyesight and a lack of financial success, he became a highly talented landscape artist, producing several illustrated travel books from around Europe.

Susan Hyman, an American art historian, has concentrated on Lear as a natural history artist, and in particular on his bird portraits. And these were something special; Lear was an innovator and a precocious one. Ceasing formal education at 15, he began visiting the newly-opened Zoological Gardens in Regent's Park, sketching the birds and

animals. Within two years he was contributing drawings for the first visitors' guidebook, and the following year, when still only 18, he began work on a most ambitious undertaking, a series of 42 large (folio) plates of parrots. The most important difference between Lear and his contemporaries - and many of his successors - was that he drew from living birds. Instead of relying solely on the stuffed skin, with the resulting unnatural and angular postures on the plate, he worked in the Zoo, persuading the keepers to catch the birds for him to make detailed notes and measurements. Not content with painting more lifelike birds than anyone else, Lear also engraved his own stones for the lithographic reproduction, rather than handing over his plates to an engraver with the inevitable loss from the artist's

intentions. It is perhaps this, above all, which gives Lear's parrots their individuality and their character, as they peer out at one with beady eye and knowing look.

Several plates from *Illustrations of the Family Psittacidae, or Parrots* are reproduced here, together with, and adding greatly to the interest, sketches and preliminary studies, showing Lear's pencil notes on colour details and shape, instructions to the colourists, and charming vignettes of different postures. And, just once or twice, somewhat disparaging sketches of visitors to the Zoo who gathered round him to watch.

Lear contributed plates to books by many famous nineteenth-century naturalists, including Jardine, Selby, Eyton and Gould. This last-named ruthlessly exploited Lear, paying him little and even erasing his name from the plates and substituting his own. That Lear knew what was happening is perhaps revealed by a sentence in a letter written to Gould from abroad some years later: "I often think of

you when I see the large kites and falcons which are so numerous in the mountains where I have been residing".

The many sides to Lear's character and his varied talents are all brought out in this book. As well as the superbly reproduced colour plates there are many in black and white, including drawings from his nonsense books and a few from his period as a landscape artist. Lear was indeed a most remarkable man, and if, for the many, his memorial is made up of the limericks and verses of nonsense, then this book should certainly add to the few for whom Lear is quite simply the finest of bird artists.

In his own words:

How pleasant to know Mr Lear!
Who has written such volumes of stuff!
Some think him ill-tempered and queer,
But a few think him pleasant enough.

M.A. Ogilvie is a Research Officer at the Wildfowl Trust, Slimbridge, working on population and migration studies, and the author of several books on ornithology.

literature on the flora of the Alps will be translated into English and German, at least. These drawbacks can then be rectified. In the meantime, this Italian edition, besides being an essential acquisition for botanical libraries, will give much added interest to those carrying in their car Bacon's *Mountain Flower Holidays in Europe* (Alpine Garden Society, 1979).

G. Pontecorvo retired in 1975 as a member of the research staff of the Imperial Cancer Research Fund, London.

Prayer for Shetland

H.N. Southern

The Natural History of Shetland. By R.J. Berry and J.L. Johnston. Pp.380. (Collins: 1980.) £8.50.

THE first author of this book is well known as a professional biologist; the second, since he is resident in Shetland and was previously the Nature Conservancy Council's representative there, has an intimate knowledge of the islands. Theirs is an unusual book because it is a mixture of a massive amount of raw data and an interpretation of some only of it, and because — in spite of this mixture — some important principles emerge from the illumination shed on the whole subject by the senior author.

Professor Berry has already shown in his previous book in this series — *Inheritance and Natural History* (Collins, 1977) — the significance, for tracking small-scale evolutionary processes, of studying the faunas and floras of archipelagoes. Shetland is such an archipelago, right on our doorstep, and he has used the information amassed, both by individual researchers and by the extensive surveys, funded by the government and the big oil companies, to further this interpretation of how the Shetland fauna and flora have evolved. The intensive investment in the extraction of North Sea oil has aroused fears of the possibility of disasters being inflicted upon a fragile environment and money has been provided to lay a base-line survey from which effects on the habitats can be measured. This survey of the fauna and flora has been a tremendous effort and its achievements can be judged from the full appendices to this book which comprise checklists for all of the groups of animals and plants that have been studied.

The text of the book is heterogeneous, as would be expected from the many authors who have taken part in the survey. Some aspects receive much fuller treatment than others (83 pages on birds and 9 on invertebrates, for example), again as might be expected. The chapters on vegetation and geology are solid stuff, though the maps illustrating the latter are reduced to the extent of needing a magnifying glass to

Photographic atlas of alpine flowers

G. Pontecorvo

I Fiori delle Alpi. By F. Rasetti. Pp.316. (Accademia Nazionale dei Lincei: Rome, 1980.) L19,500.

THIS monumental work, aimed at the naturalist, is the outcome of 20 summers of one man's intense botanizing throughout the Alps. The 572 excellent colour illustrations reproduced here — selected from some 8,000 photographs taken by the author in the wild — are one of the two major outcomes of that activity. The illustrations cover practically every species of angiosperm that has its altitudinal centre of distribution above the tree line. They include, as a most welcome but unusual feature, all 61 of the high-altitude Gramineae, Cyperaceae and Juncaceae; in fact the plates of these three families, usually neglected in illustrated field floras, are of an even higher quality than the rest.

The standard of the photographs and of their reproduction in this book disposes for good of the myth that preliminary identification from colour plates is easier if they are reproductions from paintings rather than photographs. The idea is that it is only in paintings that relevant diagnostic features can be emphasized and shown to best effect. Yet, of course, it all depends on the knowledge of the photographer and his ability to bring out what is botanically desirable. Rasetti includes an interesting discussion of this matter in a chapter on alpine plant photography.

The second upshot of the author's devoted and painstaking work on the Alpine flora lies in the longest and by far the most valuable chapter in his book. In

this, the botany of individual valleys and massifs is considered. For each, a short geological outline is followed by a discussion of the distinctive species of the area and their distribution. Becherer, in his *Führer durch die Flora der Schweiz* (Schwabe: Basel, 1972), has already attempted something of this kind, but limited to Switzerland and a few contiguous areas. Rasetti covers all of the Alps, north to south and from the Maritimes to the Schneeberg. Furthermore, in limiting himself to the alpine and nival plants, he has been able to deal with the subject much more extensively and in great detail. This section of Rasetti's book will be of great help to botanists, ecologists and amateur orophytologists who wish to know what grows where. Even the local wizard having a very detailed knowledge of the flora of his valley — and there are many — will find how and why his local haunts differ from those of the next valley.

It is a pity that a book with these two outstanding features — and many more minor ones — should be marred by a number of editorial shortcomings. To mention four: the format and weight are such that it cannot be carried for field identification, for which, presumably, the pictures were meant; the scale of a subject must be inferred by going from the picture to the index and then to the description of the species; there is no indication, with each picture, of time, altitude and locality; the description in great detail of the topography of the Alpine Range (with some new and valuable suggestions as to subdivision) is not supported by a sketch.

No doubt this major contribution to the

discern the details. Professor Berry, however, has made a great effort to produce some unification in this pot-pourri. His general introductory chapters, together with those he has written on the mammals (including Shetland man) and other groups (whose composition and distribution shed light, under his guidance, on what Darwin called "that mystery of mysteries — the first appearance of new beings on this earth"), have a vitality that leavens the lump.

The final chapters on the practicalities of

the extraction of the oil and the precautions that have been taken against disasters and on the potential problems which conservationists, and the Shetlanders themselves, have faced and are facing are cagey. We are left with the feeling that a good deal of what could be done has been done — as for the rest, we must just pray.

H. N. Southern was formerly Senior Research Officer of the Animal Ecology Research Group in the Department of Zoology, University of Oxford.

Evolution of thinking on evolution

John Lawton

Evolution for Naturalists: The Simple Principles and Complex Reality. By P.J. Darlington, Jr. Pp.262. (Wiley-Interscience: 1980.) £12.75, \$27.50.

THIS is a lovely book — easy and enjoyable to read, outspoken, tart, philosophical and wise. In it, at all times and in whatever mood, Darlington displays a deep personal understanding of the living world, acquired by a life-time of study in the best traditions of scientific natural history, combining accurate observations with acute curiosity and a love for obscure corners of the living world, in his case carabid beetles.

It is not, or rather I did not find it, a straight-forward account of biological evolution written for interested laymen (that is, naturalists without a scientific training). Rather, it is an extremely personal view of evolution that might profitably be read by professional biologists and laymen alike.

It is perhaps trite, but it struck me forcefully whilst reading *Evolution for Naturalists* how human views of biological evolution have themselves evolved, and how they continue to do so. In the book, Darlington traces the early history of evolutionary thought, and explains carefully and precisely why Darwin's ideas are now favoured at the expense of others. Although the central theme is familiar, I was not aware of the clear and accurate statements of a theory of evolution by natural selection made by Scottish landowner and forester Patrick Matthew, 28 years before the publication of the *Origin*. Even the best ideas are not always favoured by selection until the time and place are right!

Contemporary views on evolution range from the extremely primitive (of the "it doesn't happen" fundamental religious school), to the very sophisticated — with the whole gamut scrambling for attention, acceptance and success. Darlington has his own clear ideas about which should fail and which should succeed. There are the sound principles of the modern synthesis, carefully explained: sadly, whilst finding "great flaws in it", I doubt that President-elect Ronald Reagan would have the time or inclination to inform himself properly, even if he finds the book in his stocking on Christmas morning.

Amongst those who are better informed, Darlington himself sees "hopeful monsters" rampaging through the collective consciousness: most mathematical models of evolution, for example, he assigns firmly to this pedigree. I disagree; partly because I think he misunderstands some of the models, and partly because models force people to state their assumptions. They can therefore be useful, not monstrous, even if eventually proved wrong.

Other ideas he positions explicitly or implicitly at different points along the path of scientific appraisal and selection. Some, such as the strong group-selection theme permeating the book at all levels, are not to my taste either, but look set to enjoy a period of rapid growth and attention, despite strong initial selection against them. Those falling under the broad mantle of "sociobiology" have recently grown explosively, and are now subject to hard testing on many fronts; not all of this testing, in Darlington's opinion, is either ethical or scientific. Still more ideas are murmurings in the minds of a few: Darlington firmly believes, for example, that many adaptations are far from perfect, a heretical notion if ever there was one, but cogently argued.

Philip Darlington is retired. He wrote the book because he wanted to, not because he had to. He finds it neither far-fetched nor funny that Darwin might not be allowed a first-class college education today because he was bad at mathematics and worse at languages. He writes with affection about carabid beetles on mountain tops; the role of stone-throwing in the evolution of human co-ordination, perception and intelligence; and why evolution drives a progressive increase in organic diversity. And through all this detail, he writes directly, simply and with conviction. The book often made me pause; it sometimes made me cross; but above all it made me think again about evolution in all sorts of ways. If you are interested in evolution, and if you do not get *Evolution for Naturalists* in your stocking, buy it, or borrow the new President's — he won't have time to read it, though I wish he would.

John Lawton is a Senior Lecturer in Ecology in the Department of Biology at the University of York.

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Abroad migration

John Krebs

The Mystery of Migration. Chief editor Robin Baker. Pp.256. (Macdonald-Futura/Viking: 1980.) £13.95, \$29.95.

AT FIRST glance this appears to be a straightforward popular book about migration in plants and animals. However I suspect that many lay readers might find parts of the first two chapters sufficiently difficult to dissuade them from reading the rest.

The first chapter is an introduction consisting largely of an historical survey of ideas about bird migration. It includes two especially charming engravings from sixteenth- and seventeenth-century natural history books. One shows Swedish fishermen on the ice hauling in nets loaded with swallows; at that time it was thought that swallows spent the winter months

underwater. The other describes the ontogeny of a barnacle goose, showing how it develops from a goose barnacle through a series of gradual transitions in an illustration worthy of M. C. Escher.

After this the going gets a bit tough. Chapter 2 works through a series of definitions — lifetime track, familiar area, exploratory instinct, preferred direction, partial migration, navigation, orientation and piloting — and develops some quite complicated arguments that would be more appropriate to a textbook than a popular account. An example is the section on the arbitrariness of various definitions of habitat. Although the diagrams here and elsewhere in the book are outstanding, the absence of labelling often leaves the reader confused — I struggled for some time, without success, to understand the graph about prairie dog migration on p.29.

The reward for persisting beyond the second chapter is a taxonomically arranged survey of migration (plants, invertebrates, insects, fish, amphibians and reptiles, various mammals and Man). The pages are full of interesting information and many are illustrated by some fine photographs and diagrams. My personal favourite is a photo of the trunk of a pine tree completely clothed in roosting monarch butterflies. The term "migration" is used in its broadest possible sense in the book so that it includes, in addition to "conventional" examples such as salmon, birds and butterflies, "migration" by potatoes or shallots as a result of forming swollen underground stems. The rationale for this extraordinarily broad definition is developed in Baker's earlier book (*The Evolutionary Ecology of Animal Migration*; Hodder and Stoughton, 1978). He argues that all forms of movement serve the same function (to transfer the organism from one site to another, even if sites are separated only by centimetres), so all should be labelled as migration.

The book has been written by a team of authors, and this may have contributed to some problems in continuity. For example, on p.32 the point is made that tracking radar allows an observer to follow individual birds of known species on their migration, while on p.135 we are told that radar studies are limited by their inability to distinguish between species of migrating birds. The possibility that pigeons might find their way home by infrasound is referred to in a general chapter on p.27, but warrants no mention at all in the section devoted to a detailed discussion of navigation and homing in birds on pp.161–165.

An inexperienced reader may find the text a bit difficult, but beginners and old hands alike cannot fail to admire and enjoy the photographs and — lack of labelling apart — the diagrams. □

John Krebs is a Lecturer in Zoology at the Edward Grey Institute of Field Ornithology, Department of Zoology, University of Oxford.

Companions for the fungal foray

Clark T. Rogerson

Mushrooms of Western North America. By R.T. Orr and D.B. Orr. Pp.293. (University of California Press: 1980.) \$6.95. *Mushrooms Wild and Edible.* By Vincent Marteka. Pp.290. (W.W. Norton: 1980.) \$15.95.

Mushrooms of Western North America is only the second guide to cover an area until now without much published information on its fungi. The area dealt with is the Rocky Mountains west to the Pacific Coast and from the Mexican boundary north to include Alaska. It is an introductory book written primarily for the novice in mushroom lore and identification, and treats only some of the commonest species. The introduction gives concise information on characteristics of fungi, seasonal occurrence and habitat, mycorrhizal associations, collecting fungi, and on edible, toxic and hallucinogenic species. The scientific nomenclature follows modern usage, about 30 of the larger ascomycetes, 100 non-gilled basidiomycetes and 200 or more gilled mushrooms being included. The descriptions are brief, appear to be accurate and include microscopic details. Information on edibility is given. Keys are given for the larger genera. Perhaps the major fault is that less than a third of the species are illustrated in colour, making the identification of the others by descriptions alone difficult for the beginner.

As an introductory field guide to the commonly found species, the Orrs' book will be useful and certainly should stimulate a greater interest in the fungi of the western United States and Canada.

Marteka's book, which deals with some common and easily recognized mushrooms, has several attributes that make it unique amongst the recent crop of mushroom books. First, it is arranged by seasons during which the mushrooms occur in the wild — not by a system of classification of those fungi included; second, it contains a wealth of information on folklore, first-hand field observations, environmental variations, case histories of experiences in eating mushrooms — much more than is typically found in books of this kind; third, it includes up-to-date and new information on preparing mushrooms for eating, both fresh and preserved, stalking wild and not so wild mushrooms in stores, growing your own mushrooms, and a good section on further reading. Only 30 species are treated in detail; they are described briefly but correctly, and all are illustrated with good colour photographs and additional black-and-white photographs and drawings are provided for some species.

The book is well written and organized: it really is enjoyable to read and browse through. While written primarily for

mushroom hunters in the United States, the choice of species will also make it useful in other parts of the world. For an introduction to the mushrooms and for a companion book to others which cover more species, it can be recommended to all who are interested in the subject. □

Clark T. Rogerson is at The New York Botanical Garden, Bronx, New York.

Love of hands

Bernard Wood

Hands. By John Napier. Pp.176. (George Allen & Unwin/Pantheon: 1980.) £12.50, \$13.95.

AS FAR as I am concerned there are two categories of books, "work" books and "non-work" books. I wish I could say that I read too many "work" books and too few "non-work" books, but the truth is that I don't read enough of either kind. Reviewing "work" books is simple in theory. They are either research monographs, symposium volumes or textbooks. The criteria are usually clear. Do they advance the subject? Are they comprehensive? Are they up to date? Mercifully, I am never called upon to review "non-work" books, but to take me past the first few pages they have to be entertaining, in the broadest sense, or illuminating, and preferably a combination of the two. John Napier's new book *Hands* straddles my literary boundary in an interesting way.

The book is presented in two nearly equal parts. The first deals with the structure and evolution of the hand. It sets out to explain what human hands are like, what they can do and how they came to be the way they are. The second part is devoted to four separate aspects of the hand. It covers how we use our hands, and considers tool-using and gestures. It also examines the forensic use of fingerprints, and "handedness". The author's aim is made clear in the first chapter. He wants to pass on to the reader something of the fascination and beauty of the hand, and he seeks to communicate some of the wonder we all feel when we come across any example of near-perfect harmonization of structure and function.

John Napier is less than fair to himself in the opening pages. He declares that he is

The Image of Eternity: Roots of Time in the Physical World by David Park, reviewed in *Nature* 288, 305; 1980, is available in the UK through Transatlantic Book Service, 24 Red Lion Street, London WC1, price £8.

infatuated with the hand. In my dictionary infatuation is defined as "an extravagantly foolish or unreasoning passion". I think his treatment neither extravagant or unreasoning, but, like all lovers, he occasionally fails to appreciate that what interests him may not always interest those not so closely involved, and vice versa. For non-anatomists, particularly, the section on morphology and evolution may be too dense, but elsewhere it is difficult to avoid making the charge that some subjects are treated too superficially. Again and again, just when I was beginning to be fascinated, the chapter was drawn rapidly to a close and my curiosity was left unsatisfied. The sections on fingerprints and handedness, for example, left me wanting more. The discussion of handedness hardly touches upon the neurological concomitants and there was no discussion of lateralization or speech. The neurological control of the hand is dealt with in the book, but very briefly. Too many questions are left unanswered. For example, does the brain deal with individual muscles, or whole movements? I was excited by the broader perspectives offered by his treatment of topics such as palmistry, the etymology of vernacular and anatomical terms, and the problems besetting a nail-brush designer! I am only sorry that he chose to explore so few of these general fields of interest.

On a more academic level, there are, of course some points of detail where I would take issue with the author, but these are few. I wonder why he discounts the pisiform as a carpal bone (p.35), and the course of the radial artery depicted in Fig.20 is an unusual one to say the least. Stone artefacts are found with the fossil remains of *Australopithecus* (p.113), and the mean brain sizes quoted for early hominids are confusing in their exactness (p.166). These minor points of detail, however, do not detract from a text which is generally of a high standard. The use of technical terms is sometimes uneven, and perhaps the publishers could have served the author better if they had encouraged him to include a glossary. I was disappointed, too, by the illustrations; they seemed predictable and rather dull.

For some years now there has been a steady buzz on the anatomical/anthropological "grapevine" to the effect that John Napier was writing a monograph on the hand. Many of us were eager to see Wood Jones's classic monograph of the 1920s updated. Unfortunately, while *Hands* will entertain a much wider audience, it will disappoint the professional fraternity. Not because it is not a good book in its own way, but because it is not the sort of book we were hoping for. I only hope that *Hands* will have whetted the author's appetite sufficiently for him to go on and produce the book we are still waiting for, and which he is particularly well equipped to write. □

Bernard Wood is Reader in Anatomy at The Middlesex Hospital Medical School, London.

Flair for fossils

John C. W. Cope

The Natural History of Fossils. By C. R. C. Paul. Pp.292. (Weidenfeld & Nicolson/Holmes & Meier: 1980.) Hardback £15, \$37.50; paperback £6.95.

WHEN an experienced palaeontologist draws upon his knowledge and adds his own views on diverse aspects of modern palaeontology, the result promises to be interesting. When this is combined with a flair for imparting information to the uninitiated such as Dr Paul has, the result is a book which is informative, clearly presented and easy to read.

This is no textbook of palaeontology — nor does it profess to be such. Rather, it explains what palaeontology is concerned with — the uses of fossils, their palaeobiology, their ecology, their evolution and so on. Those who seek any sort of taxonomic treatment of fossils must look elsewhere.

In his discussion of each new concept, the author makes sure his readers are never left behind; there is careful exposition of many non-palaeontological topics, including "way-up" criteria, cyclothem, radiometric dating, palaeomagnetism, osmosis. Although, therefore, the book is readily accessible to the layman, it contains much that any aspiring palaeontologist should know.

From his introduction, the author goes on to discuss fossilization, with a useful review of trace fossils. Then follows treatment of sediments, fossil communities and associations. Consideration of faunal provinces and continental drift brings in palaeotemperature studies. The chapter on growth studies covers moult stages and modern work on growth-line studies of bivalves and corals. There is a useful

assessment of the adequacy of the fossil record and subjects such as the origin of life, evolution and the development of life on land are clearly discussed.

In his attempt to make the book comprehensible to the layman, Dr Paul has sacrificed some of the information which would make the volume more compulsory reading for undergraduates. Thus some diagrams are used to illustrate basic non-palaeontological concepts, whereas the space available and the subject really merit the imparting of additional and more advanced information through the medium of the diagrams. Incidentally, the reproduction of those diagrams included is not of the highest standard — how much the rough textured paper is responsible for this is difficult to judge.

There are 40 photographs which illustrate some of the subjects covered in the text. Their quality varies and they seem rather an afterthought — I do not feel that they contribute greatly to the book, but a layman unused to seeing fossils might well disagree.

In a book which is so stimulating and in which the author has clearly taken considerable care in presentation of his material, it is strange to find some words consistently spelt incorrectly. I found particularly jarring the mis-spellings of "Trias", "Lias", "Gault" and "isostasy".

The other titles in the *World Naturalist Series* suggest that this is primarily a book for the layman. As such, an introduction to modern ideas in palaeontology, it is a clearly presented exposition of the subject which deserves to succeed. □

John C. W. Cope is a Senior Lecturer in Geology at the University College, Swansea.

Geological tour de France

John Sutton

Geology of France (with twelve itineraries). By Ch. Pomerol et al. Pp.256. (Masson: 1980.) £8, \$25.

GEOLOGY came into existence as a science in large part through the travels across France and Britain of some remarkable observers who were active more than 150 years ago. The founding fathers of geology are long since dead, but the rocks from which they learnt survive; with modern motorways we can cross in an afternoon what Smith, Murchison, de Beaumont and Cuvier took weeks to explore on horseback or on foot.

Geological traverses, though no substitute for painstaking mapping, can be great fun. Over there we see the hilltop of

Jurassic rock that Vercingetorix held against the Romans; here we find the Tertiary deposits overlying the Chalk from which all authentic Champagne is produced; and down there huge blocks of rock were displaced in the geological past to open the caves where Roquefort cheese now matures. This is what this book is about. It is not a geology of France as the English title wrongly states; it provides an account, prefaced by a summary of the geological structure of France, of what one can find of geological interest and much else besides on 12 journeys through the French countryside. "France géologique, grands itinéraires", as the original has it, puts the matter much better. Professor Pomerol assumes a knowledge of geology

on the part of the reader and uses technical terms freely. This and the remarkably bad indexes — erratic to the point of fascination — may restrict use of the book to those who already have a pretty good idea of what they are looking for and who know the jargon. This is a pity, for this guide is laid out in a way which could reward more than the committed geologist and could enliven a long journey across the country or contribute to an enjoyable leisurely holiday tour. Of the 12 itineraries, five radiate from Paris, though none reaches the Channel coast, so the English-speaking visitor descending at Charles de Gaulle airport fares better than the cross-Channel sailor. Other routes take in the French Alps, the Pyrenees and the Massif Central.

Instructions are specific, give route numbers, indicate good spots to park and are thoroughly practical. With their aid the

traveller can take in the general geology of the passing countryside and supplement this with stops to see outcrops at first hand.

This is a book to have handy in the car or to read by the Christmas fireside in anticipation or in retrospect. The translation is adequate but no more. With a little more trouble, fewer technical terms, an accurate index, a route or two for the traveller entering France at the Channel coast and some reference to relevant books in English the translation could be much improved. The Anglo Saxon critic must in fairness reflect that, Canada apart, geological guides in French to North America and Britain are rare birds. In this spirit we should welcome this migrant from the south and hope it reappears in finer plumage. □

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Seismology explained

Peter J. Smith

Earthquakes. By G. A. Eiby. Pp.209. (Heinemann Educational/Van Nostrand: 1980.) £7.50, \$14.95.

AT SCHOOL more than 20 years ago, I was pleased to come across a newly-published little book on earthquakes, written by G. A. Eiby of the Seismological Observatory in Wellington, New Zealand. Like most popular science books of the time — what few there were, that is — it was visually rather less appealing than the back of the local gasworks; and despite its dealing with the Earth, an international body if ever there was one, it had an irritatingly parochial emphasis in its chosen seismic examples, clearly reflecting the author's chief area of study. Still, earthquake-curious beggars in those days had little opportunity to be choosers as far as popular expositions were concerned and, for all its (really rather minor) faults, the Eiby of 1957 was a godsend to seekers of extra-curricular science.

By contrast, the 1967 revised edition of *Earthquakes* was a bit of a disappointment. It came at a bad time, of course, for never before had there been a period during which geophysics had undergone such rapid change. It must have appeared to Eiby at the time that the seismological advances of the early 1960s warranted an enlarged volume; but in retrospect it was not very wise to try to set down the state of knowledge about earthquake phenomena during a revolution in thinking in which seismics were to play an important part. Thirteen years on from there, however, things are quite different. Geophysics in general and seismology in particular continue to advance, but much more

slowly. It is now possible to take a more sober look at what the revolution of the 1960s and early 1970s threw up.

Those familiar with Eiby's two earlier volumes will be inclined to see the 1980 version as a third edition under a new publisher; and indeed there are some minor echoes of the past in chapter headings, the occasional diagram and the odd turn of phrase. In general, however, Eiby Mark 3 is really a new book — largely rewritten, glossier than its predecessors, fatter and with pages double the size. There is still a tendency to overemphasize the seismic position of New Zealand; but the context in which earthquakes may be discussed is now so much wider and more varied that this hardly matters. What does matter is that the end product, though obviously not scientifically complete in the sense that it records everything known about earthquakes, is comprehensive in that it covers authoritatively all those aspects of the subject likely to interest the non-seismologist.

So how does the new Eiby compare with its competitors? Very well. Although the layperson now has access to more material on earthquakes than ever before, most of it is tied up in wider ranging books on catastrophic phenomena — books with such exotic titles as *Disasters*, *Geological Hazards*, *Forces of Nature*, *Catastrophe* and *Earthquake*. Popular books devoted solely to earthquakes are still surprisingly rare. Of the two most recent volumes within this genre, the less said the better about *The Earthquake Handbook* by Peter Verney (Paddington Press, 1979). But much more importantly there is Bruce Bolt's *Earthquakes: A Primer* (Freeman,

Earthquake damage in Concepcion, Chile, May 1960.

1978), a curiously underproduced book but splendid in content. Bolt's primer is likely to remain the natural resort of the non-seismological Earth scientist wanting to swot up on basic seismic matters, but the genuine layperson will probably find the 1980 Eiby the more attractive. □

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To the planets and beyond

David W. Hughes

ASTRONOMY has always been a subject that can be tackled at many different levels. Diversity is one of its fascinations and is illustrated perfectly by the first two books in this year's Christmas collection. Both are about planets, both consider planets in considerable detail, but amazingly there is virtually no overlap between them.

Let us start with *Planetary Geology* by John Guest with Paul Butterworth, John Murray and William O'Donnell (David and Charles, £7.95). Half of the book is made up of photographs, half of text. I state this with some certainty because throughout the volume there are photographs from the United States National Aeronautics and Space Administration on the right-hand pages and a descriptive text on the left. The Moon, Mars and Mercury are dealt with in detail and there is a brief discussion of the features of Phobos, Deimos, Venus and the satellites of Jupiter. This format is most useful as you are invariably reading about surface features which are illustrated on the opposite page and which can be seen by a flick of the eye. The text stresses the physical and chemical processes that produce rocks and landforms, and also the evolution of planetary surfaces as a function of time. Each double-page spread covers a specific topic, a few *ad hoc* examples being crater rays, faulting, lava channels and tubes, graben, fretted terrain and so on. The book is fascinating; anyone interested in planetary science will find it most rewarding. Astronomers have suffered an invasion of geologists; our cohabitation is reasonably harmonious and this book will go a long way to make the relationship even more beneficial.

The systematic pattern of planetary exploration started with the ground-based telescope and progressed by using imaging systems on flyby and orbiting spacecraft, unmanned soft landers and, in the case of the Moon, a manned landing and return of samples. Paul Doherty, in his *Atlas of the Planets* (Hamlyn/McGraw-Hill, £6.95/\$16.95), has taken us back to the beginning and gives us a succession of drawings of the planets as seen by him through his 419mm and 254mm reflecting telescopes. Doherty derives an enormous satisfaction from planet-watching and this enjoyment and enthusiasm radiates from every page of his book. The great beauty of the planets is graphically described. This is an ideal book for the amateur astronomer and is a superb introduction to the problems, joys and possibilities of studying planets with small telescopes.

The next book takes us to the boundaries of the Solar System. *Out of the Darkness: the Planet Pluto* by Clyde W. Tombaugh and Patrick Moore (Stackpole: Harrisburg/Lutterworth, \$14.95/£7.95) describes one of the most painstaking

searches in the history of astronomy, the 25-year quest for Percival Lowell's "Planet X". Tombaugh gives an autobiographical account of his childhood on the farm, his first telescopes, his amateur telescope making, and the way he approached V.M. Slipher at Flagstaff and got a job at the Lowell Observatory. We then read of the planet hunting ("a slippery business" according to Tombaugh), the life at the observatory, the new 33cm objective lens, the hours of careful telescope guiding, the succession of 35.6 by 43.2cm photographic plates, the work at the Blink-Comparator, and the search for the ninth planet. The discovery of Pluto on February 18th 1930 and its aftermath is described vividly. After the excitement dies away, comes the slow realization that Pluto is not Planet X. The search continues. More plates, more stars, more comparisons, all to no avail. In the words of Kuiper: "What Tombaugh did not find out there was even more important than what he did discover". A Jupiter-like planet, if it exists, would have been detected out to a distance of 470 astronomical units, Earth could have been detected out to 100AU, other planets like Pluto do not appear to exist out to 60AU. If Pluto isn't Planet X and there are no more planets what did Lowell do wrong? How do we explain the perturbations of Neptune? This is a great story, told by a great astronomer and makes compulsive reading.

Beyond the planets we have the stars and again two completely different books aimed at the amateur star gazer. *Star Maps for Beginners* (Simon and Schuster; \$4.95, £2.95) by I.M. Levitt and Roy K. Marshall is a competent introduction to the splendours of the night sky which is now in its twenty-sixth printing. The kernel of the book is a sequence of monthly evening star charts

drawn for a latitude of 40 degrees North (Philadelphia, Denver, Peking and Madrid). The message of the book is "get outside and star gaze". For the more armchair-minded astronomer who specifically wishes to know the mythological associations of the constellations we have *The Constellations: How They Came To Be*, by Roy A. Gallant (Four Winds: New York/Scholastic: London, \$11.95/£5.25) published in late 1979. Gallant takes us on a legendary tour of the star lore of ancient civilizations and reveals the details behind the names of stars and star groups.

Turning to the more active amateur we have *The Pocket Guide to Astronomy* (Simon and Schuster/Mitchell Beazley, \$5.95/£3.95) by Patrick Moore and *An Amateur Radio Telescope* (Pachart Publishing: Tucson, \$6.95) by G.W. Swenson Jr. Moore's handy little book is ideal for the observer in a hurry. It has a full series of star and moon maps and a guide to the accessible double stars, variable stars, open clusters, globular clusters, nebulae and galaxies to be seen from both hemispheres.

Have you ever wanted to build your own radio telescope? If you haven't, read Swenson's book and you probably soon might; if you have, read it again and it tells you how. Swenson is thorough, all the information is there for the construction of an instrument relying on solid state circuitry. The book is made up of a series of articles originally published in *Sky and Telescope*. If you want to add words such as superheterodynes, baluns, yagis, impedance bridges and radio spectral pollution to your vocabulary it is just the book for you. □

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Cosmic contemplation

John Maddox

Cosmos. By Carl Sagan. Pp.416. (Random House: 1980.) \$19.95. To be published in the UK in spring 1981 by Macdonald-Futura, £12.50.

CARL Sagan is professor of astronomy at Cornell University and has for the past two years been working on a series of television films being shown, or to be shown, by English-language television stations on both sides of the Atlantic.

With the title *Cosmos*, the films add up to what the television people describe as a "blockbuster". The model seems to be that of Dr Jacob Bronowski's series *The Ascent of Man* — one man's view of an important theme.

This is very much the book of the television series. In a curious way, it reads like television. It is a series of purple passages linked together, so to speak, with magenta. Those who read the book — and there will be a great many readers, for it is a popular book — learn much less than they might have expected about the way in which the real Universe is constructed and much more than they need to know about Carl Sagan's own way of regarding the phenomenon of his own place in the scheme of things, nicely summarized by the opening sentences:

The Cosmos is all that is or ever was or ever will be. Our feeblest contemplations of the Cosmos stir us — there is a tingling in the spine, a catch in the voice, a faint

sensation, as if a distant memory, of falling from a height. We know we are approaching the greatest of mysteries.

As if in a distant memory, you can hear the television producer shouting "Fade music; voice over!"

The theme is thus that the Universe is very large, too large for the human imagination to comprehend. By great feats of imagination, humans (Sagan's word; mercifully, he is sparing with "Man") have nevertheless been able to comprehend a little. Yet the mystery remains.

The plan of the book is simple, but conditioned by its link with a sequence of film scripts. It is an explanation of the Cosmos, only partly a history of it. Sagan's special twist is his repeated statement of his well-known position on extraterrestrial life — there are living things out there somewhere but they won't look like plants or people.

For my taste, too many of the tales that Sagan tells have obvious origins in television's insatiable need for visuals. The Japanese legend of the Heike, the belief that the warriors killed in battles fought in antiquity now walk the sea bottom as crabs, is a nice way of illustrating how artificial selection works. I haven't seen the films, but would bet that Sagan and a film crew spent some time filming the carapaces of crabs in Japan.

As the title suggests, the book touches all the bases — indeed it does so economically, packing into its 400-odd pages an account of stellar evolution, galaxy formation, quasars, nucleogenesis, the origin of life, evolution and the question "Where will it all end?". In passing, Sagan gives thumbnail sketches of the contributions of people as different in character and time as Thales, Democritus, Leuwenhoek and Darwin to the progress of science.

Occasionally he uses the technique of lapsing into fiction: "Sometimes in my fantasies I imagine there was someone who thought like this . . .". There follows a soliloquy by a creature living in the "childhood of the genus *Homo*" on such matters as the economy (and the danger) of the hunter/gatherer society, the discovery of fire and the meaning of the stars.

I don't know if the stars are camp fires in the sky or holes in a skin through which the flame of power looks down on us. Sometimes I think one way, sometimes I think a different way.

Sagan's fantasy is fair enough, I suppose, even when he has this early hominid say that he would like to visit those camp fires in the sky. But the technique is a trick for making the tale more vivid, not for making it complete.

As an exercise in popularization, *Cosmos* courageously aims at the widest audience. Its readers will come away with a vivid impression that the Cosmos is an interesting place, the exploration of which has hardly begun, with a rich store of

anecdotal information about a variety of topics and with "a tingling in the spine". Some of the images are nevertheless unhelpful — is "an explosion in a spaghetti factory" the best image for the interior of a

cell, for example? I'm not sure, but I'd quite like to see how the film crew tackled that.

John Maddox is Editor of Nature.

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Carl Sagan

Heavenly images

Philip Campbell

Galaxies. By Tim Ferris. Pp.192 (Thames & Hudson/Sierra Club: 1980.) £20, \$75.

THIS book has been written for a popular audience, though professional astronomers — including galactic specialists — will probably derive some interest and not a little pleasure from it. In particular, it contains the most beautiful set of astronomical coloured photographs that I have encountered.

Tim Ferris, a science writer by profession, presents a clear and comprehensive discussion of the current understanding of his subject, working outwards, as it were, from our own neighbourhood. He discusses the structure and the constituents of our own galaxy — the stars, globular and open clusters, supernova remnants, interstellar clouds — before proceeding to the local group of galaxies and then to a more general discussion of galactic structure and evolution. Chapters are also devoted to interacting galaxies and to clusters of galaxies. The book ends with a brief and fairly standard introduction to big-bang cosmology.

In his style of writing, Ferris attempts to stimulate the reader's visual imagination to the full. A particular example of this is the

imaginary journey through the Universe with which he prefaces and parallels his chapters. Picturing the reader in a spacecraft constantly accelerating from the Earth at 1g, the author introduces the notions of relativistic time-dilation and cosmological space-time distances, his narrative occasionally tinged with wit.

At times, however, the style becomes obtrusive. Although uncluttered by references and mathematical or quantitative details, the text is scattered with similes and metaphors, at times laboured. Occasionally, astrophysical processes are described in the active present tense with anthropomorphized stars, for example, as protagonists. There is also a tendency to gush; the Andromeda galaxy M31, for instance, is described as "heart-rendingly" beautiful.

Such excesses apart, the informative text and superb illustrations make this an unusually fine book. The author makes full use of the large format (13" × 14") and the price of £20, though high, is for once not unreasonable. □

Philip Campbell is an Assistant Editor of Nature.

Beauty in the tools of science

Silvio A. Bedini

Antique Scientific Instruments. By Gerard L'E. Turner. Pp.168. (Blandford/Sterling: 1980.) Hardback £3.95, \$12.95; paperback \$6.95.

SCIENTIFIC instruments were once the prized possessions only of Renaissance princes and prelates, acquired for their cabinets of curiosities. Generally they were masterworks of the goldsmith and engraver, more decorative than practical, for it was not until the early seventeenth century that instruments became functional as tools of measure and observation. It was with the emergence of the mathematical practitioners and the practical sciences of mapmaking, navigating, surveying and science teaching that the craft of the instrument maker developed

and flourished. With the advent of the scientific revolution other scientific apparatus and instruments of increasing precision were required, and played an important role in the advancement of the sciences.

Now, centuries later, these early instruments are eagerly sought by museums and collectors alike. In museum exhibitions they represent the work of important artists and craftsmen, and reflect the state of the sciences as part of national history. For the collector they are evidence of romantic aspects of the past and are tangible reminders of pioneering scientists. Instruments are sought and collected by some because of their beauty, by others through intellectual curiosity.

Until the past decade, few books had been published about instruments. Then, one by one, popular works started to appear, some describing and illustrating only pieces of the finest museum quality as examples of art, and others reflecting the role of the instruments as tools of the sciences. Only one or two have been designed to inform the collector.

In *Antique Scientific Instruments* Gerard Turner addresses himself specifically to the collector and has produced the first practical handbook on the subject. He has adopted the three broad categories into which the instrument-makers themselves divided their products — mathematical, optical and philosophical — and has further segmented the mathematical instruments into five groups based on their functions — astronomy and time-telling, navigating, surveying, drawing and calculating, and weights and measures.

The instruments categorized in each of these groups are briefly described as regards their history, function and physical appearance. Under optical instruments are included microscopes, telescopes and a whole range of optical "toys" such as mirrors, camera lucida, magic lanterns, zygoscopes, stereoscopes, kaleidoscopes and cameras. The philosophical instruments covered are basically teaching devices related to mechanics, magnetism, pneumatics, hydraulics, electricity, heat and meteorology. Each of the sciences to which each group relates is described in a short history. Also included are a chapter on "Practical Advice on Collecting", a useful bibliography for further reading, a list of museums having collections of instruments and a comprehensive index.

The author is particularly well qualified to produce such a book. The Senior Assistant Curator of the Museum of the History of Science at Oxford University, which contains what is considered to be the finest collection of scientific instruments in the world, Turner is internationally recognized as an authority on the subject. He

is the author of numerous articles and several books on the subject.

This handbook is equally useful for historians and students of science, as well as for the collector and the dealer in antiquities. □

Silvio A. Bedini is Keeper of Rare Books at the Smithsonian Institution, Washington DC, and specializes in the history of scientific instrumentation and horology.

Doing time

Derek de Solla Price

Greenwich Time. By Derek Howse. Pp. 254. (Oxford University Press: 1980.) £7.95, \$24.95. *The Book of Time.* Consulting editor Colin Wilson. Pp. 320. (Westbridge Books/David and Charles: 1980.) £10.50, \$24.95.

SINCE the dawn of mankind time has had such a fascination for us that it is not much of an exaggeration to say that it has been the ultimate cause of Babylonian, Greek, and therefore of all modern science. Sheer workaday astronomical time has an exciting and crucial institutional history, and it is quite a feather in the cap of the British that Greenwich rather than Paris Observatory time became a world standard and the prime point of reference, not just for astronomers, but also for navigation and world geographers as well as the standard for the railroad age.

Derek Howse, an accomplished scholar and historical sleuth of the National Maritime Museum in Greenwich, has told the fascinating story of how it all happened in the international meetings and councils that had to deliberate the issues and resolve the intense international rivalries. The story of the International Meridian Conference, Washington 1884, is especially interesting for an analysis of the power play as convoluted as anything in the UN today. He has taken the matter right up to the present-day technologies that enable us to chide the wobbly earth for its non-uniform meanderings and institute "leap seconds" to correct it. A very pleasing book this is, written by somebody who really knows something. Not so with *The Book of Time*.

I imagine it all must have started with consultant editor Colin Wilson, a well-known novelist and author, being excited (as was J. B. Priestley) with the psychic mysteries of time and precognition. Unlike Priestley he did not write all of the book himself, but seems to have sold the publisher and editor on the idea of getting together a team of science writers to take on the merely scientific aspects of time, such

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Royal Swedish Academy of Sciences, Stockholm

A double-barrelled air pump of about 1785, signed I.B. Haas Invt, By the King's Patent Made & Sold by J.H. Hurter, London.

as historico-theological, horological, biological, physical and geological. They are all covered passably well, except perhaps the one on the history of sundials and clocks which is trite, but one misses the passion of somebody who cares and really knows their stuff. Science writing is a tricky business and needs a certain genius to pull off with distinction, and evidently nobody cared enough to do more than get this book together as prescribed.

Since I cannot recommend it, let me say that if you are interested in the philosophical and psychological problems of time you might go back to an old but splendid thesis by M. F. Cleugh, *Time and its Importance in Modern Thought* (Methuen, 1937). And if you want something more modern than her book, you might wade through the scholarly chapters in the series *The Study of Time*, edited by J. T. Fraser and others of the International Society for the Study of Time (Springer-Verlag, Vol. 2 1975), or the fine and authoritative treatment by G.J. Whitrow, *Nature of Time* (Penguin, 1976). □

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Mind-bending and the paranormal

David Davies

Science and the Supernatural. By John Taylor. Pp.180. (Maurice Temple Smith/Dutton: 1980.) £7.50, \$10.95.

THE late Chris Evans, a seasoned hunter of the paranormal, had one inflexible rule when in pursuit of unusual phenomena: do not pronounce on what you have seen for 24 hours — the excitement of the moment may cause you to say something that you would find difficult to retract later. Probably unaware of this rule, John Taylor went on television "live" in 1973 to act as (in his own words) "scientific hatchet man on Uri Geller". Before an audience of millions Taylor was bowled over by the metal-bending spectacular that unfolded in front of him (though he had apparently also seen it the previous evening) and has since devoted a substantial amount of time to pursuit of the paranormal.

In 1975 he published a book, *Super-minds* (Macmillan) which extolled the "Geller effect". He wrote "I have been convinced that such supernormal abilities [as Geller and some others generate] do actually occur . . . the total available evidence puts the Geller phenomenon truly into the class of the supernatural", and much more. In 1980 he tells a different story. Now, "the paranormal is totally normal. ESP is dead . . . I started my investigations with an open mind . . . on the evidence presented in this book, science has won". What manner of investigation,

what kind of evidence could cause such a complete about-face? For myself, in late 1973 I felt that maybe there really could be something that merited examination by scientists. Three years later I felt it was probably all bunk. This was a journey of steadily growing awareness that magicians have remarkable "paranormal" deceptions up their sleeves (as James Randi has continually demonstrated), that children are not averse to playing quite sophisticated tricks on grown-ups — particularly if it gets them attention — and that scientists, whilst detached observers of experiments under their own control, may have no special abilities in observing the much more complex goings-on that generally surround allegedly paranormal happenings. In short I learnt a lot about people.

John Taylor's route was very different. He argued that electromagnetism was the only possible explanation for unusual phenomena and therefore that electromagnetic measurements should be made and electromagnetic theories sought. And when nothing turned up (except from some fairly simple electrostatic explanations of the odd phenomenon) Taylor found himself forced to the conclusion that the phenomena were without substance. It is, for my taste, a circuitous and intellectually unsatisfying argument. For all that I believe his final conclusion is correct, I'm not sure that one is allowed to pronounce a subject dead simply because one's own

intellectual skills and measuring equipment cannot come to grips with it.

The consequence of Taylor's conclusion that the paranormal does not exist is that people have deceived him left, right and centre. But this book, which wanders anecdotally through the whole gamut of strange happenings, barely comes to grips at all with the question of why some people deceive and why others are deceived. Indeed, Taylor, now back in the anti-paranormal camp, is hard and unforgiving of those who adduce the sort of evidence which until recently he would have taken seriously: "There is a high level of gullibility where seeming inexplicable events are concerned. Even apparently sober people appear to become very naive after witnessing something in the heavens [UFOs] which they cannot readily explain". Five years ago, Taylor himself was writing: "Puharich has observed Geller enter an unidentified flying object . . . unhappily the film cartridge containing the essential record of this event was lost, having dematerialised within a few minutes". It surely needs more than a few null results from electromagnetic equipment to reverse positions quite so dramatically.

David Davies was Editor of Nature from 1973–1979. He is now director of Dartington North Devon Trust, a charity dedicated to rural development.

Avian delights: on the wing and on the water

John Andrews

PROFESSIONAL ornithology in the UK is fortunate in benefiting from substantial skilled amateur assistance and from a rapidly expanding public interest in birds and their place in nature. One result of this has been a steady increase in the funding available for research to determine the effects on birds of land-use changes and of individual development schemes. This is certainly the case with work on waders. Moving south from their breeding grounds each autumn, many species overwinter in our relatively mild, unfrozen estuaries, which are subject to threats from a great diversity of uses and abuses. In *Waders* (Collins, £9.50), Professor W.G. Hale draws together current knowledge on the biology of those species which occur in Britain, much of it either collected by amateurs or as a response to pressure from them, expressed through the conservation organizations.

Commencing with a general introduction to the species concerned, the author moves on to deal with habitats and adaptations, geographical distribution, mortality and evolution, breeding biology, migration and various aspects of wintering

ecology, including the important subject of estuarine carrying capacities which arises wherever a public inquiry asks the question "why can't these birds go somewhere else?" The author is particularly known for his own work on redshank, largely undertaken on the Ribble saltmarshes, themselves recently threatened by a scheme to embank and convert them to arable farmland. Complete with a substantial bibliography and index, the book is illustrated and presented to the high standard one expects from the *New Naturalist* series.

It is pleasing to find another publishing house aiming to produce works of similar quality. Some years ago Hamlyn produced a volume which came up to *New Naturalist* standards but was a little more adventurous in presentation and ventured outside the latter's self-imposed limits of the United Kingdom to look at birds of prey worldwide. Now they have produced a volume on *Seabirds: Their Biology and Ecology* (£7.50, \$14.95) written with evident enthusiasm by Dr Bryan Nelson of Aberdeen University and illustrated generously throughout the text not only with well-chosen colour and black-and-white photographs, but also with delightful drawings by John Busby. The author has put together an authoritative summary of the biology and ecology of seabirds worldwide in a relaxed and fluent style, without wasting words or taking the non-scientist out of his depth. After a general chapter which indicates the diversity of seabird species and of their biology, the reader is led to consider the problems and opportunities presented by their vast, rich and complex habitat, the oceans of the world. From there, the book turns to food resources and feeding methods and thence to breeding behaviour and biology, concluding with sections on movements and distribution, populations and human impact. Unlike waders, seabirds are known mostly at their breeding sites and so this aspect of their lives occupies about half the book as against one-twentieth of the text of *Waders*. All concerned deserve congratulations for producing a thoroughly enjoyable and instructive work at such a relatively modest price.

Because of their importance as quarry, geese have always attracted a good measure of interest in their distribution and numbers. Latterly, linked to a growing concern for their conservation, they have enjoyed a relatively large share of the bird research effort in both Europe and North America. For the last 13 years Dr Myrfin Owen has been working on goose ecology at the Wildfowl Trust and in *Wild Geese of the World* (Batsford, £15) he presents and discusses current knowledge of all 15 species and 23 sub-species. The first third of the book is occupied by species' accounts and the bulk of the very readable

text is reserved for discussion of social behaviour, movements and migration, summer and winter biology, population dynamics, and conservation and exploitation. Thanks to the research effort devoted to them, some aspects of the complex biology of geese are now understood, but much still remains to be worked out and the author ventures some stimulating speculation in these areas. The book is helpfully supplemented by line illustrations, accurate colour plates and a substantial bibliography.

Knowing what to leave out of any book is part of the art of writing and one most relevant to any book on general bird biology where there is a risk of taking all the information available, much of it pre-digested by other authors, and piling it into convenient heaps too large and generalized for comfort. The basic form of *Birds* (W.H. Freeman; hbk \$17.95, £10.60; pbk \$8.95, £4.90) is much the same as usual — geography, flight, migration and navigation, evolution, behaviour, physiology and song. The refreshing difference is that, while each is introduced by a crisp article summarizing the topic in very broad terms, there then follow several papers by different authors, focusing on one or another aspect of the subject. The papers — 25 in all — are selected from about 40 which have been published in *Scientific American* over the past 30 years. Despite the time span in which they were written and the many different authors, the whole book works well. It is surprisingly cohesive and easy to read.

Another imaginative, but less successful venture into natural history publishing is Derek Bromhall's *Devil Birds: The Life of the Swift* (Hutchinson; hardback £8.95, paperback £5.95). In 1948 Dr David Lack installed swift nest boxes in the roof of the tower of Oxford University Museum of Science to accommodate what has since become the best-known swift colony in the world. In 1975 Derek Bromhall decided to make a film of the same colony and this book is set against the background of its making. Swifts are fascinating birds by any standards. At about six weeks the youngsters leave their dark nest, plunging into sunlight and space at the beginning of a flight which may last for three years or more before they return to the colony to breed. Adapted to life on the wing, feeding on aerial plankton, swifts casually undertake movements of hundreds of kilometres to avoid bad weather, sleep in the air and sometimes even mate in flight. The fairly short text is an unpretentious and readable presentation of interesting information but the book is irritatingly over-illustrated by photographs many of which are of poor quality and add nothing to one's understanding of the subject. A pity because the idea is a good one and any attempt to



Passage peregrine, painted by Mary-Clare Critchley-Salmonson

From *Falconry in Arabia*, by Mark Allen

popularize species studies deserves success.

By contrast, the traditional formula works well in *Merlins of the Welsh Border* (David and Charles, £6.95). D.A. Orton presents a straightforward account of his own observations of one species — merlins watched over four breeding seasons, on the Welsh borders. The book isn't a scientific study but the author is a careful observer and a skilled chronicler of events who is not afraid to speculate imaginatively about unexplained aspects of the merlins' lives, so that the reader is entertained, informed and occasionally provoked. There are few illustrations, well chosen black-and-white studies which reveal the elegance and dash of their subject to perfection.

On recent estimates, Wales contains a large proportion of the total UK population of merlins and it is clear from studies undertaken elsewhere that their survival is closely linked to protection of their heather moor habitat. More work is needed. As Orton says: "the time has arrived for the ball to be passed from the observant amateur to the professional scientist" and next year this will happen with RSPB staff starting a study of merlin breeding and feeding requirements in Wales.

Scientific interest in birds of prey lies

partly in their position at the top of food chains which makes them useful indicators of the state of their environment. Emotion exerts its influence too — people are fascinated by this group of birds. Coincidentally, it was in Wales that Wilfred Thesiger made boyhood acquaintance with peregrine falcons. In his foreword to Mark Allen's *Falconry in Arabia* (Orbis, £15) he recalls the fascination they held for him then and goes on to describe the occasion 30 years ago when he fulfilled a long-standing ambition to accompany a hawking expedition into the Arabian deserts. For over a month the party travelled by camel, slept in the open, and lived on its quarry of hares and bustards. Some of his own photographs, taken at the time, are included amongst an excellent selection of illustrations. Mark Allen has written a fascinating book, blending fact and anecdote to entertain anyone who is interested in falconry, in Arabia ancient and modern or in the psychology of a people whose influence on our own lives — and thus, incidentally, on our birds — grows yearly more apparent.

John Andrews is Head of Conservation Planning at the Royal Society for the Protection of Birds, Sandy, Bedfordshire.

liberally sprinkled with personal observations. His introduction sets the stage for an informative book by giving an overview of the origin of raptors and their evolutionary history, with a smattering of ecological principles. He has tried to stick to the policy of not "malining any nor extolling their virtues" and in general has presented raptors as deserving of further study, especially as regards their ecological roles.

In Britain the peregrine falcon is associated most particularly with one man, Derek Ratcliffe. Derek originally came to my attention when, as a graduate student, I read his account of peregrines breaking their own eggs. I have since followed his writings with eagerness; their documentation of the peregrine is meticulous. His first full length book on the species, *The Peregrine Falcon* (Buteo: Vermillion, South Dakota/Poyser: Calton, Staffs; \$42.50, £12), is now ready to be savoured by professional and layman alike. The book is truly the definitive work on a species that has become a *cause célèbre* for the environmentally conscious. Ratcliffe's style, while clearly European, is generally flowing and easy to follow. Most readers will be captivated with the writing and astonished by the breadth of some of the information; the detail reveals the touch of a true professional.

Although much of the first half of the book chronicles the history of British peregrines, the latter chapters are of broader scope and discuss other populations. A better use of non-European literature might have lent additional support to some treatments. The introduction, largely of his reminiscences as a boy and early falconophile, is touching to read. It induced, in me at least, a sense of nostalgia. In subsequent chapters he discusses the falcon's relationship with mankind, its habitat, population trends, foods, breeding cycles, regulation of populations, the pesticide story and the future of the falcon in a world increasingly dominated by human beings.

One might think it odd to study a bird by use of fishing tackle, but that is precisely what Robert Nero has done in his study of *The Great Gray Owl* (Smithsonian Institution, \$17.50). Rare by any standards, this "Phantom of the North" is one of the least known and (in bird-watching circles) most sought-after birds in North America. In the past 12 years Nero has perhaps seen more than anyone and has banded 220. He attracts them with a wooden mouse retrieved by a casting rod, and nets the owl as it pounces on the lure.

Nero has no intent of documenting all available information but rather of helping the reader to share in the author's experiences. An entire chapter details the visit of one owl to Winnipeg. Others describe hunting in the snow, how to catch an owl, the nesting cycle and so on. Nero summarizes the essence of this owl with

A good year for raptors

Clayton M. White

MANY years ago I thumbed through Peter Scott's coloured key to the world's waterfowl and thought how unfortunate it was that a similar key did not exist for other groups of birds — parrots and raptors, for example. This thought was obviously shared by others, and in response has now come the masterfully done *Birds of Prey of the World* by Friedhelm Weick (Paul Parey/Collins; \$48, £15). Weick, inspired by Scott's waterfowl key format, has organized his book in much the same way. It will probably have only a restricted appeal to the general public, but should gain wide use among professionals and serious amateurs — it is not a volume intended to adorn the coffee table.

The book is divided into two sections. The first, with text in both English and German, contains keys to groups by colour, size and other field marks. The best drawings, together with descriptions, are here, and each genus is represented. Good as the illustrations are, however, I found much of this section of little practical value. How does one judge a "large" from a "rather large" bird? In my opinion, peregrine falcons have "plumage contrasting, dark and light", not "plumage brightly colored" as listed. The falconophile should love the second section: 40 colour plates of side-view figures, some 1,144 figures being

represented altogether. Immatures for almost all species are shown, and of the 670 subspecies described, 574 are illustrated. Though the book has shortcomings, they are but minor, and on balance Weick deserves hearty commendation for a job nicely executed.

In *North American Birds of Prey* (Morrow, \$29.95), William Mansell and Gary Low have elected for a more limited scope than Weick. This is a coffee table book. The text is combined with interesting, though at times inaccurate, illustrations. Many of the single-tone vignette head drawings at the beginning of each species account are far more accurate than the colour plate of the same bird. For example, hawks and falcons are noted for their sleek, hard plumage. Not so here. My veterinarian friend, after inspecting the bald eagle plate remonstrated, "Look how loose it looks! I'd treat it, it's ill!" Because of Low's artistic style, the owls tend to look better than the hawks.

Mansell's background centres around eastern Canada, and most of the birds included are those of that area. No truly western form is mentioned. The title, a misnomer, should rather be *Selected North American Birds of Prey I Have Known*.

Nevertheless, Mansell's treatment of those species he does cover is a useful synthesis of widespread data, gently and

lyrical prose:

A bird of mystery, the Great Gray Owl bears in its aloofness some of the remoteness of the vast northland; its plumage the color of lichens and weathered wood; its soft hooting, part of the wind . . .

A similarly species-specific book is *The African Fish Eagle* (Bailey Bros and Swinfen: Folkestone, Kent, £8.50) by the late Leslie Brown — sadly, Leslie died just prior to the publication of this book. Widely known for his analysis, with Dean Amadon, of birds of prey of the world, Brown's style is markedly different from that of Ratcliffe or Nero. But it is likewise eminently readable, chuck full of sardonic jabs and quaint commentary. Although Brown usually had large quantities of data, he often wrote as though the information was merely anecdotal. He then put raw data in tables, frequently without analysis. Such is the format of *The African Fish Eagle*.

There are nine chapters in all, dealing mainly with hunting techniques, territories

and population regulation, and breeding behaviour. Brown claims that what he has written here, however, is only the cream skimmed from a deep bowl of milk. His data causes him to challenge the widely held concept that food supply is the major limiting factor for most bird species, and to conclude that

in orthodox ornithological circles this is such an awful heresy . . . zoologists are so hooked on the theory that food supply is the ultimate controlling factor . . . [that] the possibility of other factors supervening is not allowed to enter their heads. They might as well be burned at the stake, if not hung, drawn and quartered.

Birds for all seasons

C.M. Perrins

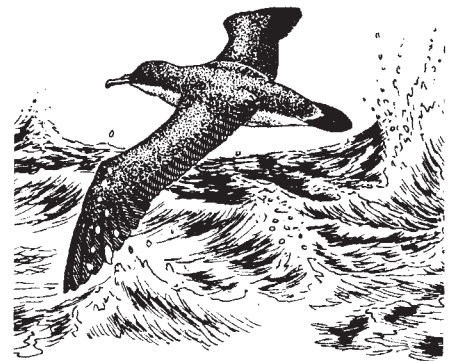
THIS review covers a number of books for the general bird-watcher plus a few more specialized works. *Collins Bird Guide: a New Guide to Birds of Britain and Europe* by G. Stuart Keith and John Gooders (Collins, £5.95) is modelled on the successful American work, *The Audubon Society Field Guide to North American Birds*. It is a soft (but hard-wearing plastic) covered, pocket-fitting guide. Almost half of the book is taken up with 613 colour photographs, the other half is text.

The pictures come two, or more usually, three to a page. They are grouped by families with a thumb index for easy location of the right group. A number of species have two (rarely more) photographs; in all, 464 species are illustrated. The two photographs are sometimes widely separated, for example amongst the wildfowl all the ducks and all the drakes are grouped together. The idea of the design is good, but the execution is less commendable. The photographs are often really not up to standard for identification purposes. The commonest fault seems to be that they have been reproduced rather poorly, many being rather dark; another objection is that some species are in highly uncharacteristic poses. The text of the book is better; there are sections on description, voice, habitat, nesting and range (including a small map); other details are added in footnotes. On the whole this section is satisfactory and some will find it a useful guide.

Michael Everett's *Guinness Book of Woodland Birds* (Guinness Superlatives, £3.95) is a nicely produced work. It starts off with some 40 pages about the distribution and history of British woodlands, the birds that occur there and the way to watch them. There is a final five-page section on the needs for conservation of our woodlands. The main part of the book covers 50 species of woodland birds. The content for each species is fairly consistent. The left-hand page includes a pen-and-ink sketch, a distribution map, notes on

He is equally critical of American scientists who travel to Africa on taxpayer's money, gather quantities of data to satisfy their goals (usually a college degree) but then never make the findings general knowledge by publishing. Outspoken? Perhaps. But this was Leslie's style. He tried to make this book reflect his feeling that birdwatching should not be a dreary statistical exercise in the arid style so unfortunately prevalent in some modern scientific writing. This, his last book, is a fitting epitaph. □

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recognition, voice, nesting and food, together with a general description of the birds' habits, relationship with man and its numbers. The right-hand page carries a colour photograph of the species concerned. These are a major feature of the book and are a little disappointing; a few seem to have lost something in the reproduction, but some others, mostly those taken by flash, could not have been very striking in the first place. All in all, this is quite a pleasing production. However, its relatively narrow scope may not appeal to all.

The British Ornithologists' Guide to Bird Life edited by J. Flegg (Blandford/Sterling; £10.95, \$27.50) is an English version of a Swedish work. It is not, strictly, a field guide, being larger with a more comprehensive text which covers the usual details of recognition, call, breeding, food, status and so on.

The most attractive feature of this book is the 128 colour plates, paintings by Staffan Ullstrom, which are refreshingly bright. Most plates cover two separate species (with varying plumages where necessary); there are small inserts of rarer species. These plates show almost all the useful features for identification without losing their attractiveness.

The text is a useful coverage of the basic information. The status sections have been adapted to Britain; even so, they are a bit

Some Christmas events

London. The Children's Centre at the British Museum (Natural History) will offer a variety of activities for children including nature quizzes, bark rubbings, microscopes to peer down and worksheets to take into the public galleries. The activities are not for children under five — or adults. Also at the Natural History Museum, the Rational Theatre Company will perform "Fossil Face — a Christmas Fantasy", the story of a prehistoric man finding himself in the 20th century. For further details telephone 01-589 6323 extn 779, 595.

The Christmas exhibition at the Science Museum is "The Mini Comes of Age" which will run until the end of February 1981. David Mosley will give a special lecture "Stage-coach to Super-train" at the Museum on five afternoons at the end of December and beginning of January. Tickets are required; for information, telephone 01-589 3456 extn 562.

New York. Among a number of attractions at the American Museum of Natural History, the Planetarium will show "Star of Wonder", a film which looks at the sky over Bethlehem at the time of the birth of Christ. Times vary and visitors should telephone in advance — (212) 724-8700 — for further details.

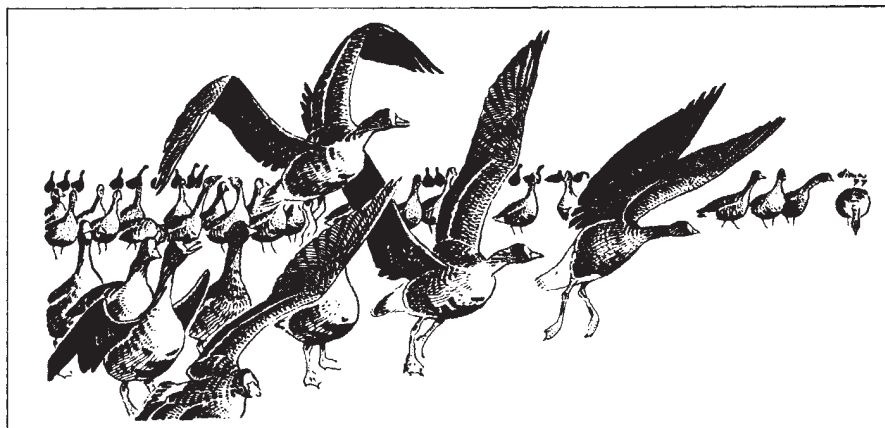
"Saturday Morning Live" at the Planetarium's Sky Theater will run through December with performances at 11.00 each Saturday. Designed for young people, each show is different; topics range from why the sky is blue to why the Moon appears to change shape.

Boston. "The Christmas Show" at the Museum of Science is for older children and adults, and relates holiday customs and traditions to astronomical events of the season. For young children, "The Winter Wishing Star" is a fairy tale which involves "a trip to the North Pole". Telephone (617) 723-2500 for dates and times.

vague and misleading. For example, "widespread but never numerous" (this for the Green Woodpecker which is very rare in northern England, practically non-existent in Scotland and does not occur in Ireland — I have been unable to work out from the text whether Ireland is supposed to be included or not.) The other main snag relates to its Swedish origin. Presumably for reasons of finance, the plates have been reproduced as in the original. Hence they include a number of birds which are very rare in Britain and some that do not occur at all. Even worse, there are a few species which occur in Britain, but are not in the book. For this reason it is difficult to recommend it highly — unless one holidays in Scandinavia.

Birds of Mountain Regions by Lars Jonsson (Penguin, £1.95) compounds the limitations of the last two books reviewed. It covers the birds of only one habitat (and a very restricted one in Britain) and, presumably again because it was set up in Scandinavia, contains many birds which do not occur here and omits some that do. Otherwise, it is attractively produced with good paintings of the birds and very cheap. It is one of a set, planned to cover all the major habitats of Europe.

The Bird-watchers A-Z by Alan Richards (David and Charles, £14.95) is a cross between a dictionary and a short-article encyclopaedia. There are seldom more than 10 items per page and not a few of the items fill about half a page. The book is attractively designed with numerous line drawings and photographs (many in colour). The text covers a wide range of ornithological subjects: "asynchronous hatching", "atlas", "Audubon", "auk", "binoculars", "bird protection acts", "bittern", "botulism" may perhaps give an indication of the breadth. Although all bird Orders are listed, only the most important bird families get a mention; there is (and is intended to be) an emphasis on Britain and Europe. In many entries up-to-date references to major relevant works are cited. No two ornithologists would choose identical items for inclusion in such a work, but on the whole the coverage is good and the contents fairly accurate. It



should certainly be useful to someone with a limited library.

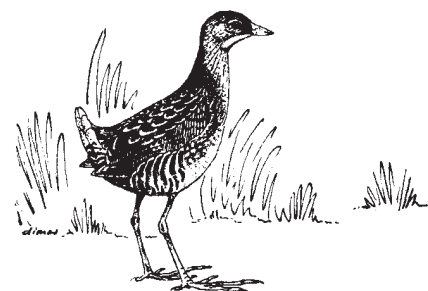
Bill Oddie's Little Black Bird Book (Eyre Methuen, £4.95) is something entirely different. It is a part-Punch, part-Potter lampoon on bird-watching. The fact that Bill Oddie is himself an enthusiastic and knowledgeable "twitcher" makes the book's thrusts cut deeper; it is an "inside job". Enthusiastic bird-watchers should enjoy it (unless they are entirely devoid of humour), but it should also appeal to others interested in that odd British trait of utter dedication to curious pursuits.

The remaining three works are rather more specialist in nature. *The Complete Birds of the World* by Michael Walters (David and Charles; £12.50, \$35.50) is a list of the 8,600 or so birds of the world. These are crammed into 325 pages of two-column text; there are therefore some 26 species per page and only very little space for each. A typical entry might be: "*Oreortyx picta* (Douglas) Plumed Quail, Mountain Quail Pacific USA from Washington to Lower California/ seeds, leaves, shoots, berries and insects/ e 10-12/1? ♀♂ 21" (the last sections mean clutch size is 10-12 and incubation, probably by both female and male, for 21 days). Not all entries are as complete as that since breeding information is not available for all species.

For many years there was no readily available check-list of the birds of the world, but in recent years there has been a spate of them. This is certainly one of the better ones. However, it has what is, to me, a fatal flaw. This relates to what one uses such a book for. I use it mainly to check the scientific name for a bird for which only the English name is given in an article, or vice versa, or to check to which family a bird belongs. Inexplicably, this book has no index except to families. So, if you want to find the scientific name of the Moustached Green Tinkerbird or the English name of *Aulacorhynchus coeruleicinctus* there is simply no way to do it unless you know the family, and even then you could have to search many columns of text (the humming birds, for example, occupy 23 columns of print). If you are thinking of getting such a

book, I would look at others. J. Gruson's *A Check-list of Birds of the World* (Collins, 1976) has less information, but it does have an index.

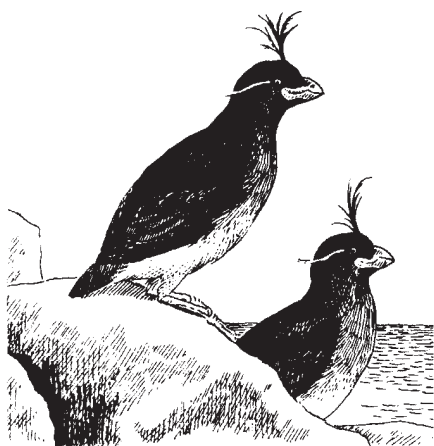
Frontiers of Bird Identification, edited by J.T.R. Sharrock (Macmillan Journals, £7.95), is a selection of articles, originally published in the magazine *British Birds*, on the identification of difficult species pairs or groups. Usually one or more of the birds



concerned is very rare in Britain. The articles have been published over a number of years and have been up-dated where necessary. The book is well produced with many black-and-white photographs and line drawings that amplify the text. For those dedicated to the identification of species in the field this will be a valuable asset.

Finally, Gerald Tuck's *A Guide to Seabirds on the Ocean Routes* (Collins, £4.50) is what its name suggests; it lists in some detail what might be seen along 25 different ocean voyages (which leave few places in the world unvisited). Each route is broken down into component parts. Seasonal variation is also covered. Although the book makes passing comments on the distinctive features of some of the commoner species, it is not intended as a work on identification; it should be used in conjunction with another book by the same author, *A Field Guide to the Seabirds of Britain and the World* (also Collins). Tuck's new book is a useful aid for travellers, but really it needs to be used in conjunction with the field guide. □

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BOOKS RECEIVED

Mathematics

- EDWARDS, B. *The Readable Maths and Statistics Book*. Pp.328. Hbk ISBN 0-04-31007-4. (Allen & Unwin: 1980.) Hbk £15; pbk £6.95.
- JACOBS, O.L.R. *et al.* *Analysis and Optimisation of Stochastic Systems*. Based on the Proceedings of the International Conference held at the University of Oxford, September 1978. Pp.574. ISBN 0-12-378680-0. (Academic: 1980.) £20, \$46.
- SELDIN, J.P. and HINDLEY, J.R. (eds). *To H.B. Curry: Essays on Combinatory Logic, Lambda Calculus and Formalism*. Pp.606. ISBN 0-12-349050-2. (Academic: 1980.) £26.60, \$61.50.
- TRAUB, J.F. and WOŹNIAKOWSKI, H. *A General Theory of Optimal Algorithms*. Pp.341. ISBN 0-12-697650-3. (Academic: 1980.) \$36.

Astronomy

- BEER, A., POUNDS, K. and BEER, P. (eds). *Vistas in Astronomy*, Vol.23. Pp.404. ISBN 0-08-026046-2. (Pergamon: 1980.) £46, \$104.
- WAYMAN, P.A. (ed.). *Highlights of Astronomy*, Vol.5. As presented at the XVIIth General Assembly of the IAU 1979. Pp.868. Hbk ISBN 90-277-1146-1; pbk ISBN 90-277-1147-X. (Reidel: 1980.) Hbk Dfl. 160, \$84; pbk Dfl. 75, \$39.50.

Physics

- BARRY, J.D. *Ball Lightning and Bead Lightning: Extreme Forms of Atmospheric Electricity*. Pp.298. ISBN 0-306-40272-6. (Plenum: 1980.) \$29.50.
- CHIEN, C.L. and WESTGATE, C.R. (eds). *The Hall Effect and Its Applications*. Proceedings of the Commemorative Symposium, held at The Johns Hopkins University, Baltimore, November 1979. Pp.550. ISBN 0-306-40556-3. (Plenum: 1980.) \$59.50.
- GIBB, T.C. *Principles of Mössbauer Spectroscopy*. Pp.254. Flexi ISBN 0-412-23060-7. (Chapman & Hall: 1980.) £8.50.
- KAUFMANN, W.J. III. (introduction by). *Particles and Fields*. Readings from *Scientific American*. Pp.139. Hbk ISBN 0-7167-1233-4; pbk ISBN 0-7167-1234-2. (W.H. Freeman: 1980.) Hbk £8.80; pbk £3.80.
- MARTON, L. and MARTON, C. (eds). *Advances in Electronics and Electron Physics*, Vol.53. Pp.322. ISBN 0-12-014653-3. (Academic: 1980.) \$39.50.
- PERLMUTTER, B. and SCOTT, L.F. (eds). *Recent Developments in High-Energy Physics*. Studies in the Natural Sciences, Vol.17. Pp.311. ISBN 0-306-40565-2. (Plenum: 1980.) \$39.50.
- READ, F.H. *Electromagnetic Radiation*. Pp.331. Hbk ISBN 0-471-27718-5; pbk ISBN 0-471-27714-2. (Wiley: 1980.) Hbk £19.50 \$58.50; pbk £7.95.
- RIAZUDDIN (ed.). *Physics and Contemporary Needs*, Vol.4. Lectures presented at the Fourth International Summer College at Nathiagali, Pakistan, June 1979. Pp.587. ISBN 0-306-40474-5. (Plenum: 1980.) \$65.
- USLENGHI, P.L.E. (ed.). *Nonlinear Electromagnetics*. Pp.426. ISBN 0-12-709660-4. (Academic: 1980.) \$30.
- WILKINSON, D. (ed.). *Progress in Particle and Nuclear Physics*, Vol. 3. Pp.334. ISBN 0-08-025020-3. (Pergamon: 1980.) £31.
- WOOLLEY, R.G. (ed.). *Quantum Dynamics of Molecules: The New Experimental Challenge to Theorists*. NATO advanced Study Institutes Series, Vol.57. Series B: Physics. Proceedings of the NATO Advanced Study Institute held at Trinity Hall, Cambridge, UK, September 1979. Pp.557. ISBN 0-306-40462-1. (Plenum: 1980.) \$59.50.

Chemistry

- BOCKRIS, J. O'M., CONWAY, B.E. and YEAGER, E. (eds). *Comprehensive Treatise of Electrochemistry*, Vol.1: *The Double Layer*. Pp.453. ISBN 0-306-40275-0. (Plenum: 1980.) \$49.50.
- KEMP, D.S. and VELLACCIO, F. *Workbook to Accompany Organic Chemistry*. Pp.421 + *Solutions Manual for Organic Chemistry* Pp.250. Loose leaf ISBN 0-87901-124-6. (Worth: New York, 1980.) Np.
- MENGER, F.M. and MANDELL, L. *Electronic Interpretation of Organic Chemistry: A Problems-Oriented Text*. Pp.216. Hbk ISBN 0-306-40391-9; pbk ISBN 0-306-40379-X. (Plenum: 1980.) Hbk \$27.50; pbk \$12.50.
- PRYOR, W.A. (ed.). *Frontiers of Free Radical Chemistry*. Based on papers from a symposium held at Louisiana State University in April 1979. Pp.385. ISBN 0-12-566550-4. (Academic: 1980.) \$27.
- RHYS-WILLIAMS, A.T. *Fluorescence in Liquid Chromatography*. Pp.86. (Perkin-Elmer: 1980.) Flexi £3.95.

Technology

- BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE. *Energy in the Balance*. Some Papers from the Annual Meeting of the BAAS held at Heriot-Watt University, Edinburgh, 1979. Pp.234. Flexi ISBN 0-86103-031-1. (IPC Science and Technology Press: Guildford, Surrey, 1980.) £9.

- HORVÁTH, C. (ed.). *High-Performance Liquid Chromatography: Advances and Perspectives*, Vol.1. Pp.330. ISBN 0-12-312201-5. (Academic: 1980.) \$35.

- IKEDA, S. *et al.* (eds). *Statistical Climatology*. Developments in Atmospheric Science, 13. Proceedings of the 1st International Conference held at the Inter-University Seminar House, Hachioji, Tokyo, November 1979. Pp.388. ISBN 0-444-41923-3. (Elsevier Scientific: 1980.) \$68.25, Dfl.140.

- KAZMERSKI, L.L. *Polycrystalline and Amorphous Thin Films and Devices*. Pp.304. ISBN 0-12-403880-8. (Academic: 1980.) \$37.50.

Computer Science

- ROHL, J.S. and BARRETT, H.J. *Programming via Pascal*. Pp.327. Hbk ISBN 0-521-22629-7; pbk ISBN 0-521-29583-1. (Cambridge University Press: 1980.) Hbk £12.50; pbk £5.95.

Earth Sciences

- BISWAS, M.R. and BISWAS, A.K. (eds). *Desertification*. Associated Case Studies prepared for the United Nations Conference on Desertification. Environmental Sciences and Applications Series, Vol.12. Pp.523. ISBN 0-08-023581-6. (Pergamon: 1980.) £33.50, \$75.
- BRINDLEY, G.W. and BROWN, G. (eds). *Crystal Structures of Clay Minerals and their X-ray Identification*. Mineralogical Society Monograph, No.5. Pp.495. ISBN 0-903056-08-9. (Mineralogical Society: London, 1980.) £28, \$70.
- EDMONDS, E.A., WILLIAMS, B.J. and TAYLOR, R.T. *Geology of Bideford and Lundy Island*. Geological Survey of Great Britain: England and Wales. Pp.143. ISBN 0-11-884120-3. (HMSO: 1979.) Np.
- LILLIE, A.R. *Strata and Structure in New Zealand*. Pp.450. (A.R. Lillie: 58 Gordon Mansions, Torrington Place, London, 1980.) £8.50 plus £1 postage.
- TROEH, F.R., HOBBS, J.A. and DONAHUE, R.L. *Soil and Water Conservation for Productivity and Environmental Protection*. Pp.718. ISBN 0-13-822-155-3. (Prentice/Hall: 1980.) £16.20.

Biological Sciences

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- ANDERSON, J.W. *Bioenergetics of Autotrophs and Heterotrophs*. The Institute of Biology's Studies in Biology, No.126. Pp.59. Flexi ISBN 0-7131-2807-0. (Edward Arnold: 1980.) Np.
- ARDLEY, N. *Illustrated Guide to Birds and Birdwatching*. Pp.197. ISBN 0-7063-6002-8. (Ward Lock: 1980.) £6.95.
- BARRASS, R. *The Locust: A Laboratory Guide*. 3rd Edn. Pp.74. Flexi ISBN 0-435-60100. (Heinemann Educational: 1980.) £2.50.
- BOURNE, G.H. and DANIELLI, J.F. (eds). *International Review of Cytology*, Vol.67. Pp.337. ISBN 0-12-364467-4. (Academic: 1980.) \$35.
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- FARMER, J.N. *The Protozoa: Introduction to Protozoology*. Pp.732. Flexi ISBN 0-8016-1550-X. (C.V. Mosby: 1980.) Np.
- FURTH, A.J. *Lipids and Polysaccharides in Biology*. The Institute of Biology's Studies in Biology, No.125. Flexi ISBN 0-7131-2805-4. (Edward Arnold: 1980.) £2.60.
- FRISTROM, J.W. and SPIETH, P.T. *Principles of Genetics*. Pp.687. ISBN 0-632-00647-1. (Blackwell Scientific: 1980.) £11.50.
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- HOLLIDAY, P. *Fungus Diseases of Tropical Crops*. Pp.607. ISBN 0-521-22529-9. (Cambridge University Press: 1980.) £55.
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SUCKLING, K.E. and SUCKLING, C.J. *Biological Chemistry: The Molecular Approach to Biological Systems*. Pp. 381. Hbk ISBN 0-521-22852-2; pbk ISBN 0-521-29678-1. (Cambridge University Press: 1980.) Hbk £25; pbk £9.95.

WILSON, P.J. *Man, The Promising Primate: The Conditions of Human Evolution*. Pp. 185. ISBN 0-300-02514-9. (Yale University Press: 1980.) £8.80.

Applied Biological Sciences

OSOL, A. *et al.* (eds). *Remington's Pharmaceutical Sciences*. 16th Edn. Pp. 1928. ISBN 0-912374-02-9. (Wiley: 1980.) £35.

PERRY, T.W. *Beef Cattle Feeding and Nutrition*. Pp. 383. ISBN 0-12-552050-6. (Academic: 1980.) \$35.

Psychology

ARGYRIS, C. *Inner Contradictions of Rigorous Research: Organizational and Occupational Psychology*. Pp. 203. ISBN 0-12-060150-8. (Academic: 1980.) \$16.

HANSEL, C.E.M. *ESP and Parapsychology: A Critical Re-evaluation*. Pp. 325. Hbk ISBN 0-87975-119-3; pbk ISBN 0-87975-120-7. (Prometheus: Buffalo, New York, 1980.) Np.

McMILLAN, J.H. (ed.). *The Social Psychology of School Learning*. Pp. 262. ISBN 0-12-485750-7. (Academic: 1980.) \$21.

VAN PRAAG, H.M. *et al.* (eds). *Handbook of Biological Psychiatry, Part III. Brain Mechanisms and Abnormal Behaviour — Genetics and Neuroendocrinology*. Pp. 400. ISBN 0-8247-6965-1. (Dekker: 1980.) SwFr. 62.

Sociology

KAHN, J. and WRIGHT, S.E. *Human Growth and the Development of Personality*. 3rd Edn. Pp. 227. Hbk ISBN 0-08-023383-X; flexi ISBN 0-08-023382-1. (Pergamon: 1980.) Hbk £14.95, \$33.50; flexi £6.95, \$15.50.

MALMBERG, T. *Human Territoriality. Survey of Behavioural Territories in Man with Preliminary Analysis and Discussion of Meaning*. New Babylon. Studies in the Social Sciences, 33. Pp. 346. Flexi ISBN 90-279-7948-0. (de Gruyter: Berlin and New York, 1980.) DM 75.

History of Science

FINOCCHIARO, M.A. *Galileo and the Art of Reasoning. Rhetorical Foundations of Logic and Scientific Method. Boston Studies in the Philosophy of Science*, Vol. 61. Pp. 478. Hbk ISBN 90-277-1094-5; pbk ISBN 90-277-1095-3. (Reidel: 1980.) Hbk Dfl. 80 \$42; pbk Dfl. 40 \$21.

FROMM, E. *Greatness and Limitations of Freud's Thought*. Pp. 147. ISBN 0-224-01875-2. (Cape: 1980.) £4.95.

Anthropology

CHARLES-DOMINIQUE, P. *et al.* *Nocturnal Malagasy Primates: Ecology, Physiology, and Behaviour*. Pp. 215. ISBN 0-12-169350. (Academic: 1980.) \$27.50.

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ALFONSECA, M. *Human Cultures and Evolution*. Pp. 136. ISBN 533-03747-6. (Vantage Press: New York, 1979.) Np.

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HARMES, J. *The Magic of Herbs and Flowers. An Illuminated Manuscript Celebrating their Healing Properties*. Pp. 70. ISBN 0-333-29375-4. (Macmillan: London, 1980.) £2.95.

ISAACS, J. (ed.). *Australian Dreaming: 40,000 Years of Aboriginal History*. Pp. 304. ISBN 0-7018-1330-X. (Merewether: New York, 1980.) Np.

LURKER, M. *The Gods and Symbols of Ancient Egypt: An Illustrated Dictionary*. Pp. 142. (Thames & Hudson: 1980.) £8.95.

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O'RIORDAN, T. and TURNER, R.K. (eds). *Progress in Resource Management and Environmental Planning*, Vol. 2. Pp. 246. ISBN 0-471-27747-9. (Wiley: 1980.) £16.75.

PAULY, M.V. (ed.). *National Health Insurance: What Now, What Later, What Never? A Conference Sponsored by the American Enterprise Institute for Public Policy Research*. Pp. 381. Hbk ISBN 0-8447-2184-0; pbk ISBN 0-8447-2185-9. (AEIPPR: 1150 17th Street, NW, Washington DC, 1980.) Pbk \$8.25.

SALT, B. *Rural Science 2. Cassell's Rural Science Series*. Pp. 153. Flexi ISBN 0-304-30425-5. (Cassell: 1980.) £2.50.

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WHITTOW, J. *Disasters. The Anatomy of Environmental Hazards*. Pp. 411. Flexi ISBN 0-14-02-2114-X. (Penguin: 1980.) £4.50.

WINTER, R. *The Scientific Case Against Smoking*. Pp. 125. Hbk ISBN 0-517-539470; pbk ISBN 0-517-541416. (Crown: New York, 1980.) Hbk np; pbk \$4.95.

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● Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.

● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

● Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.

● 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

Articles should be accompanied by an abstract of not more than fifty words. Letters should begin with a paragraph giving the background and main conclusion in terms intelligible to as wide a readership as possible.

Manuscripts may be submitted either to London or New York. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should be identified in the margin on their first appearance. Equations should occupy single lines if possible: $\exp(a)$ is preferred to e^a if 'a' is more than one character.

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Abbreviations should follow the *World List of Scientific Periodicals*, fourth edn (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated 'Personal communication' and 'unpublished' should be incorporated in text.

Artwork should be sent with the manuscript and clearly marked with author's name and the figure number. Line drawings should be either photographic prints or in Indian ink on heavy cartridge paper, tracing paper or similar materials. Most figures are reduced to one column width so originals should be about as wide as a page of *Nature*. To enable figures, particularly maps, to be edited in the same style as the text, they should contain only essential material. Ideally, an unlettered original and three lettered copies should be provided; labelling on halftones should, if possible, be avoided entirely. Magnifications quoted should be for the figures as submitted. We are always glad to see artwork for possible use on the cover, but cannot guarantee its return.

In order to save on postal expenses we return only the top copy and artwork of manuscripts that we cannot publish.

Nature's publishing policy is outlined in 258, 1 (1975) and 264, v, 11 Nov. (1976).

ANNOUNCEMENTS

Awards

Georges Köhler (Basle Institute of Immunology) has been awarded the Albion O. Bernstein M. D. Prize for 1980 by the New York Medical Association.

Dr Günther Ohloff (Switzerland) is to receive the 1980 Hackforth-Jones Award for his work in perfumery chemistry.

Dr Arthur Kornberg, (Stanford University) has been awarded the 1981 Forteza Gold Medal by the Institute for Cell Research of the Caja de Ahorros de Valencia, Spain.

In the Chemical Writers' Awards BASF in association with the SCI have announced that **Peter Grange** (technical editor of *British Plastics & Rubber*); is Chemical Writer of the Year; **Mike Hyde** (editor, *Chemical Insight*) is Chemical Reporter of the Year; **John Murphy** (editor, *Plastics Industry Europe*) is Chemical Application Writer of the Year; and **David McDonald** (editor, *International Pest Control*) is Agrochemical Writer of the Year.

Appointments

Trevor W. Cole has been appointed the P. N. Russell Professor of Electrical Engineering at the University of Sydney, Australia.

Dr J. L. Hall, reader in plant physiology in the University of Sussex has been appointed to a Chair of Biology in the University of Southampton with effect from 1 April 1981.

New members of the Directorate of the CERN Laboratory in Geneva include: director general, **Professor H. Schopper**; research directors, **Professor R. Klapisch** (France), **Dr Erwin Gabathuler** (UK); technical director, **Dr G. Brianti** (Italy); LEP Project Leader, **Dr E. Picasso** (Italy); administrative director, **Dr R. F. Heyn** (Netherlands).

Meetings

13-16 April, **88th Health Congress**, London (Royal Society of Health, 13 Grosvenor Place, London SW1, UK).

29-30 April, **Gauge Theories of the Fundamental Interactions**, London (The Royal Society, 6 Carlton House Terrace, London SW1, UK).

4-7 May, **Emerging Environmental Solutions for the Eighties**, Los Angeles (Institute of Environmental Sciences, 940 East Northwest Highway, Mt Prospect, Illinois 60056).

6-7 May, **Monoclonal Antibodies and Ultrasensitive Immunoassay in Human Diagnosis and Monitoring of Therapy**, Gardone Riviera (RIA 81, Fondazione Giovanni Lorenzini, Via Monte Napoleone, 23, 20121 Milan, Italy).

8-9 May, **Calcitropic Hormones Methods and Clinical Applications**, Gardone Riviera (RIA 81, Fondazione Giovanni Lorenzini, Via Monte Napoleone, 23, 20121 Milan, Italy).

10-16 May, **The Structure and Development of the Greenland — Scotland Ridge**, Bressanone (Prof. Svend Saxov, Laboratory of Geophysics, Aarhus University, Finlandsgade 6-8, DK-8200 Aarhus N, Denmark).

14 May, **Ores in Sediments: Mineralising Fluids**, London (Geological Society of London, Burlington House, London W1, UK).

11-15 May, **Remote Sensing of Environment** Ann Arbor (Ann Arbor Conference & Visitors Bureau, 207 East Washington St, Ann Arbor, Michigan 48106).

14-18 May, **Reproductive Immunology**, Banff (Dr T. G. Wegmann, Division of continuing Medical Education, 12-103 Clinical Sciences Bldg, University of Alberta, Edmonton, Alberta, Canada T6G 2G3).

18-22 May, **Ground based Spacecraft Flight Dynamics Operations**, Darmstadt (R. E. Münch, esoc, Robert-Bosch-Strasse 5, 6100 Darmstadt, FRG).

19-21 May, **Telecommunications Energy Conference**, London (IEE, Savoy Place, London WC2, UK).

1-13 June, **Methods of Immunological Research and Diagnosis**, Buffalo (James F. Mohn, The Ernest Witebsky Center for Immunology, State University of New York at Buffalo, 210 Sherman Hall, Buffalo, New York 14212).

2 June, **Geothermal Studies**, Southampton (Geological Society of London, Burlington House, London W1, UK).

8 June, **High Pressure as a Reagent and an Environment**, Washington DC (Dr Robert S. Shane, 7821 Carreigh Parkway, Springfield, Virginia 22152).

10-12 June, **Leukotrienes and Other Lipooxygenase products**, Florence (Fondazione Giovanni Lorenzini, Via Monte Napoleone 23, 20121 Milan, Italy).

16-17 June, **Chromatography in Biochemistry, Medicine and Environmental Research**, Venice (Dr A. Frigerio, Mario Negri Institute for Pharmacological Research, Via Eritrea, 62-20157 Milan, Italy).

18-19 June, **Mass Spectrometry in Biochemistry, Medicine and Environmental Research**, Venice (Dr A. Frigerio, Mario Negri Institute for Pharmacological Research, Via Eritrea, 62-20157, Milan Italy).

New ventures

A new service has been launched by Harwell which makes the Laboratory's neutron beam facilities available to industry for research into materials problems. The service, based on Harwell's DIDO and PLUTO materials testing reactors, provides a range of techniques — radiography, crystal diffraction, small angle scattering and vibrational spectroscopy — for the investigation of materials properties. Further details from: Peter Schofield, Materials Physics Division, AERE Harwell, Didcot, Oxon OX11 0RA, UK.

A new company Merseyside Laboratories has been formed to manufacture and distribute peptides, amino acid derivatives, resins, anti-sera and reagents and catalysts for asymmetric synthesis. Merseyside Laboratories also has a custom peptide synthesis service. This company is the UK and European distributor of peptides manufactured by Peninsula Laboratories, California. Further information from: Merseyside Laboratories, PO Box 62, 21 Aston Court, Kingsland Grange, Woolston, Warrington WA1 4SL, UK.

London-based Scientific and Technical Book Services Ltd have acquired world distribution rights in English for two Chinese scientific journals. *Science in China* (Scientia Sinica) and *Science Bulletin* (Kexue Tongbao). Further information from: Jennifer Clarkson, Gordon & Breach, 41/42 William IV St, London WC2N 4DE, UK.

The *Journal of Psychophysical Systems* commences publication shortly, with the object of promoting research into the behaviour of systems in which the presence of psychic factors cannot be ignored. Further information from: Prof. D. F. Lawden, Dept of Mathematics, University of Aston, Gosta Green, Birmingham B4 7ET, UK.

Elsevier announces a new bi-monthly publication *Applied Catalysis*, an international journal devoted to catalytic science and its applications. Sample copies from: Elsevier Scientific Publishing Company, PO Box 330, 1000 AH Amsterdam, The Netherlands.